CRANFIELD UNIVERSITY

GRIVIN CHIPULA

Optimising nutrient potential from compost and irrigation with wastewater to meet crop nutritional requirements

School of Applied Sciences

PhD
Academic Year: 2009 - 2012

Supervisors: Dr Ruben Sakrabani
Dr Tim Hess

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ABSTRACT

Globally agricultural production is facing serious challenges to provide adequate food supply to meet a growing population. However, the reduced capacity of soil to support and sustain agricultural production as a result of soil fertility decline is impacting negatively on agricultural growth. Increase in the price of inorganic fertilisers and limited availability of nutrients from organic amendments has reduced progress in improving soil fertility. This research therefore aims at contributing knowledge towards evaluating the maximisation/optimisation of nutrients in compost and secondary treated sewage effluent (STSE) amended soils to meet the nutritional requirements of crops for sustainable crop production and environmental protection. STSE was irrigated on soils (sandy loam and clay loam) amended with greenwaste compost in soil incubation, glasshouse/pot and lysimeter studies. Perennial ryegrass *(Lolium perenne)* was grown in the pots and lysimeter studies. The incubation experiment showed that for a clay loam, N mineralisation in treatments with STSE alone and combinations of compost and STSE was higher than the applied N. Increasing compost quantity in compost and STSE nutrient integration resulted in reduced net N mineralisation in the clay loam soil. In the sandy loam, increasing compost contribution in compost and STSE nutrient integration resulted in an increase in net N mineralisation. Cation exchange capacity, microbial diversity, quality of available carbon and drying and rewetting cycles influenced the net nitrogen mineralisation dynamics in both soil types. Increasing the contribution of STSE while reducing compost quantity resulted in increased nitrogen use efficiency and ryegrass dry matter yield. The environmental threat to ground and surface water pollution through NO_3^--N leaching may be enhanced by the inclusion of STSE in integrated compost and STSE nutrient supply to plants. Similarly, the threat to eutrophication due to phosphorous leaching is likely to be higher with integration of compost and STSE. Ryegrass dry matter yield reduced with increasing compost contribution while the concentration of N in ryegrass herbage for the combinations of compost and STSE was above the minimum requirement for N in herbage for productive grazing and dairy cattle in the pot experiment. Using compost and STSE of similar characteristics, the ideal approach to maximise nutrient potential from compost through irrigation with STSE is when 25% compost is integrated with 75% STSE with respect to nitrogen supply.

Keywords: Nutrient integration, irrigation, compost, effluent, nitrogen mineralisation

To Tiffany and Mary

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
C	Carbon
CEC	Cation Exchange Capacity
Cu	Copper
CUSTP	Cranfield University Sewage Treatment Plant
Cd	Cadmium
Cr	Chromium
C/N	Carbon to Nitrogen ratio
°C	Degree Celsius
CO_2	Carbon dioxide
DM	Dry Matter
DM_{yield}	Dry Matter Yield
D.o.F	Degrees of Freedom
dS m^{-1}	Decisiemens per meter
EC	Electrical Conductivity
EU	European Union
ET	Evapotranspiration
Ext P	Extractable Phosphorous
FAO	Food and Agriculture Organisation
FISP	Farm Input Subsidy Program
GDP	Gross domestic product
Ha	Hectare
K	Potassium
KCl	Potassium Chloride
kg	Kilogram
LSD	Least Significant Difference
Ltd	Limited
l	Liter
Pb	Lead
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
MAFF	Ministry of Agriculture, Fisheries, and Food
Mg	Mega gram
mm	Millimetre
me/l	Milliequivalent per litre
mg	Milligram
MDG	Millennium Development Goals

mol L^{-1}	Moles per liter
NO_3^--N	Nitrate nitrogen
NH_4^+-N	Ammonium nitrogen
Ni	Nickel
N	Nitrogen
N_{uptake}	Plant uptake of nitrogen
N_m	Mineral N
N_o	Potentially mineralisable N
NUE	Nitrogen Use Efficiency
N_2O	Nitrous oxide
NM_{net}	Net nitrogen mineralisation
PAS	Publicly Available Specification
PO_4^{3-}	Phosphate
P_{uptake}	Plant uptake of phosphorous
P	Phosphorous
P_{plant}	Phosphorous in plant material
PFP_e	Partial factor productivity
SOM	Soil organic matter
SAR	Sodium Adsorption Ratio
SEM	Standard error of the mean
SS	Sum of squares
SMN	Soil mineral nitrogen
STSE	Secondary treated sewage effluent
µS cm^{-1}	Microsiemens per centimetre
TN_{plant}	Total nitrogen in plant material
TN_{soil}	Total nitrogen in soil
TP_{soil}	Total Phosphorous in soil
TC_{comp}	Total Carbon in compost
UK	United Kingdom
UNESCO	United Nations Education, Scientific and Cultural organisation
UNDP	United Nations Development Programme
WHO	World Health Organisation
Zn	Zinc

1 INTRODUCTION

This chapter outlines the contribution of agriculture to developing economies. It discusses factors impeding sustainable agricultural growth of which soil fertility decline is one of them. Shortfalls associated with usage of organic amendments and limitations of inorganic fertilisers have also been presented. The description of the problems associated with soil fertility decline forms a background to the research study whose aim, objectives and outline methodology are stated at the end of the chapter.

1.1 Research background

1.1.1 Current challenges in agriculture and food security

Globally agricultural development remains fundamental to food security, economic growth and poverty reduction. Growth of agricultural productivity over the last decade has been driven by extensive use of chemical fertilisers, irrigation water, agricultural machinery and pesticides (Pretty, 2008). But extensive use of chemical fertilisers has contributed to environmental pollution and it has been hampered by the rapid increase in prices. Therefore, the desire to feed the booming world population has been the driving force to research in soil fertility and ways of enhancing crop production.

It is anticipated that 50 to 70 million people will be added annually to the world population until the mid 2030’s (FAO, 2008). Most of this increase is expected to take place in developing countries especially the group of 50 least developed countries (FAO, 2008). It is projected, for example that the African population will reach about 490 million between 1995 and 2020 at a population growth rate of about 2.4% (African Fertiliser Summit, 2006). Predictions show that by 2030 the world will need to produce 50% more food and energy, together with 30% more available fresh water, whilst mitigating and adapting to climate change (Beddington, 2009). With limited resources, improving resource efficiency in usage of organic and inorganic soil amendments will be vital to feed the increasing world population.

Despite the significant agricultural contribution in world economies, agricultural growth has stagnated. While per capita food production has increased in Asia and Latin America by 76 and 28% respectively, Africa has fared badly, with food production per person 10% lower than in 1960 (Pretty, 2008). Africa was not part of the Green Revolution hence it was left behind in the race to enhance crop productivity and yield. Most of the experience of Green Revolution technology and its impact has been outside Africa and relatively little empirical evidence exists within African communities (Terry, 2012). Actually, if Green Revolution technology were to be applied to staple production, consumers and producers would benefit and 70 million Africans would be lifted out of poverty (Diao et al., 2008). That explains why eradicating extreme hunger and poverty is one of the top goals of the Millennium Development Goals (MDG) that has a target of reducing by half the proportion of people suffering from hunger. Whilst progress has been made in other parts of the world (e.g. East Asia), Sub-Saharan Africa is among the regions not expected to achieve the poverty reduction target (UNDP, 2012). The reduced capacity of soil to support and sustain agricultural production will likely contribute to failure to meet the target.

There is an urgent need for intensive research on sustainable intensification of agricultural production i.e. producing more food from the same area of land while reducing environmental impacts (Godfray et al., 2010; Deeks et al., 2013; Pretty, 2002). According to Pretty et al., (2008) sustainability in agricultural systems centres on the need to develop technologies and practices that do not have adverse effects on environmental goods and services, are accessible to and effective for farmers and lead to improvements in food productivity. Sustainable land management technologies have potential to generate private benefits for farmers, by improving soil fertility and structure, conserving soil and water, enhancing the activity and diversity of soil fauna and strengthening the mechanisms of elemental cycling (Branca et al., 2013).

The decline in soil fertility and land degradation are some of the factors contributing to chronic food shortages. Other than declining soil fertility, climate change coupled with rising fuel costs and production uncertainties are the other two main criteria to threaten food production (McKelvey and Marshall, 2007). The decline in soil fertility over the years has affected crop production as soil fertility is a very important aspect of soil productivity. Soil fertility decline is not only about nutrient deficiency; it is also a problem of physical and biological degradation of soils, inappropriate crop varieties and of pests and diseases (Swift and Shepherd, 2007). However, nutrient deficiency is one of the predominant factors influencing soil fertility decline.

Application of organic amendments, amongst others forms the backbone of agricultural sustainability due to the less adverse impacts to the environment. But the gradual N release from the soil organic-N pool and the low N mineralisation rates affect crop yield when organic amendments e.g. compost alone is applied as a source of crop nutrients. The end result is that high crop yields are often associated with increased compost application rates. Integrating readily available sources of inorganic-N with compost can reduce the amount of compost applied. Reduction in quantity of compost applied also reduces labour costs associated with compost making, transportation and application of the compost. According to Sikora et al., (2001), if compost is applied to agricultural land at the N requirement of grain crops (40–100 kg N ha^{-1} crop requirement); application rates approach 40–100 Mg ha^{-1} compost. If compost is integrated with for example, sewage effluent, less quantity of each nutrient source can be applied thereby limiting the exposure to sewage effluent by farmers and reducing the labour demand associated with making compost and transportation costs.

1.1.2 Compost application to arable land

The art and science of making compost for use as fertiliser has been around since ancient times. Composting reduced in the twentieth century as the use of chemical fertilisers increased, particularly following World War II (Epstein, 1997). The application of chemical technology (e.g. fertilisers) to increase agricultural productivity was attributed to be a better solution due to rapid availability of nutrients to meet crop nutrient requirements and subsequently, higher yield.

In most developed countries, composting is seen as a method of diverting organic waste materials from landfills while creating a product, at relatively low-cost, that is suitable for agricultural purposes (Eriksen et al., 1999; Wolkowski, 2003). This trend may be attributed to economic and environmental factors, such as municipal landfill capacity; costs associated with landfilling and transportation of materials; adoption of legislation to protect the environment; decreasing the use of commercial fertilizers; increasing the capacity for household waste recycling and improved quality of compost products (Hargreaves et al., 2008).

Composting in developing countries is essentially done to provide plant nutrients as a replacement to inorganic fertilisers. Compost application enhances nutrient availability and organic matter status of the soil (Parkinson et al., 1999). Compost has a suppressive effect toward several plant pathogens (Litterick et al., 2004).

Although various site-specific factors (e.g. compost maturity, composting conditions, climate, soil properties and soil management) may affect N-dynamics in compost amended soils, the short-term availability of N to plants is minimal since the majority (>90%) of total compost N is bound to the organic N-pool (Amlinger et al., 2003). N derived from compost amendments mainly acts through the soil organic N pool (Gutser et al., 2005) as the mineral N content of compost is low due to nutrient losses during composting (Hao et al., 2004; Tiquia et al., 2002). The low N mineralisation from compost result into huge quantities of compost applied to provide enough N for plant uptake. This practice can lead to addition of excesses of other nutrients and trace elements (Hargreaves 2008). Novel and innovative approaches are therefore required to improve nutrient availability from compost and reduce the quantity of compost required to meet the nutritional requirement of plants.

Combined application of organic amendments and inorganic fertiliser has gained recognition as one of the appropriate ways of addressing soil fertility depletion (Chivenge et al., 2011). As discussed earlier on, unaffordability of inorganic fertilisers is a likely limitation to the adoption of this innovation. Hence, other options of combining compost with sources of readily available nutrients may be viable alternatives to the integration of compost and inorganic fertilisers e.g. combination of compost and effluent. This approach will not only allow nutrient maximisation in compost but also provide an opportunity to recycle effluent. This thesis is attempting to contribute knowledge towards an understanding of the combined application of compost and STSE in order to supply plant nutrients. This will be a tool for nutrient optimisation in compost and waste water recycling for sustainable crop production whilst at the same time ensuring environmental protection from the leaching of nutrients and heavy metals.

1.1.3 Waste water recycling in agriculture

Agriculture is the major water use in the world. It accounts for 70% of total global withdrawals (Kayikcioglu, 2012). In developing countries, about 95% of the total water withdrawal is for agriculture with the demand projected to continue to increase (FAOWATER, 2008). About 20 million hectares of agricultural land is irrigated with untreated, partially treated wastewater or river water polluted by wastewater (World Water Assessment Programme, 2009). However, wastewater irrigation accounts for only 1% of total agricultural water use (Kayikcioglu, 2012). Surface water pollution problems can be reduced by reusing wastewater thereby conserving valuable water resources and providing the nutrients contained in the wastewater to grow crops, which in turn provides economic benefit for farmers by using less chemical fertilisers.

Despite the numerous benefits of recycling STSE for crop production can cause environmental risks to farmers, consumers and the environment. Irrigation methods used to apply the STSE, crops under cultivation, management and harvesting practises used are known to influence the transmission of diseases (Fonseca et al., 2007a). But in most developing countries, the national burden of diseases from wastewater irrigation is just a fraction of that resulting from continuing poor access to safe drinking water and adequate sanitation, and poor hygiene standards (World Water Assessment Programme, 2009). Wastewater recycling in agriculture provides a justification for the high investment costs for wastewater treatment in many developing countries.

Irrigation with sewage effluent provides a means to supply both moisture and nutrients to plants. However, because untreated sewage effluent may contain heavy metals, high levels of nutrients and pose a health hazard, STSE can be ideal as it is either disposed in rivers or into the ground (Qadir et al., 2010). Irrigation with treated effluent can stimulate microbial activities thereby increasing the microbial population in the soil (Fonseca et al., 2007a). Microorganisms are responsible for decomposing soil organic matter and nutrient mineralisation.

Recycling of wastewater in some instances has resulted in excessive inputs of elements with adverse impact on plants. For example, the high total N of reclaimed water from secondary treatment makes it unfavourable for crop growth (Chiou, 2008). Excess vegetative growth, lodging, delayed maturity and reduced fruit quality are some of the consequences of excessive nutrient supply. Meanwhile, accumulation of heavy metals in the soil due to waste water irrigation has been reported. Heavy metals are non-biodegradable and can persist in the environment long enough to diminish soil quality and to be taken up by plants and up the food chain, posing a major threat to soil quality and biodiversity (Katanda et al., 2007).

Combining wastewater/effluent and compost can therefore provide an opportunity to reduce the total loading rate of the wastewater in soil. This can reduce excessive supply and accumulation of nutrients and heavy metals in the soil. In combination with compost, STSE will readily provide plant nutrients as the compost mineralisation is slow but instrumental in improving soil structure.

1.2 Project description

The research project critically reviews available literature on nutrient integration between organic amendments and other sources of readily available nutrients. Previous research on nutrient integration has focused solely on compost and inorganic fertiliser as such there is little or no information available about the proposed nutrient integration of compost and STSE. This research aims at filling existing knowledge gaps about compost and sewage effluent integration as a source of plant nutrients and water thereby offering a solution to problems of soil fertility decline and water recycling.

1.2.1 Research aim

The aim of this research is to contribute knowledge towards evaluating optimisation of nutrients in compost and sewage effluent amended soils to meet nutritional requirements of crops for sustainable crop production and environmental protection.

1.2.2 Objectives

To achieve the overall aim of the research project, the following objectives were developed:

i. To determine the mechanism of interaction of nutrient dynamics associated with irrigation of STSE and compost on soils.
ii. To evaluate the effects of final sewage effluent irrigation on soils amended with compost on ryegrass production and leaching of nutrients.
iii. To assess the fate of nutrients and heavy metals in the soil as a result of STSE irrigation on compost amended soils.

iv. To identify the ideal approach to optimising nutrient potential from compost through irrigation with STSE.

The following hypotheses were tested in this research study;

Hypothesis 1

Crop production (dry matter) will be influenced by a unit increase (25%) in the contribution of STSE in integrated compost and STSE nutrient application.

Hypothesis 2

The proportion of STSE in combined application of compost and STSE will be the major determining factor for leaching of nitrate and phosphorous. The readily available nitrate and phosphorous from STSE will be susceptible to leaching.

Hypothesis 3

Accumulation of plant nutrients and heavy metals in the soil profile will be affected by the quantity of STSE in integrated compost and STSE nutrient application.

Hypothesis 4

The optimum compost and STSE nutrient integration will be combinations with less compost (25%) as compared to the contribution of STSE (75%).

1.3 Outline methodology

In order to comprehensively meet the aim and objectives of the research project, the research methodology was sub-divided into 3 categories; (i) incubation experiments (ii) pot (glasshouse) experiment (iii) lysimeter experiment. Each of the experimental units was designed to contribute to the overall aim and objectives of the research. The experiments were designed to be in synergy with each other. Information, knowledge and lessons learnt from one experiment were used in the subsequent experiment.

Incubation study: The incubation study focused on understanding N dynamics, potential N mineralisation and microbial biomass N and C as influenced by the combinations of compost and STSE. This study was conducted under laboratory conditions in an incubator at Cranfield University (Soil Laboratory). This allowed for variables such temperature and soil moisture to be controlled. Soils were sampled once every 30 days over a period of 120 days and analysed for microbial biomass and soil process rates (N mineralisation).

Glasshouse study: The glasshouse pot experiment was set up in a semi-controlled environment at Cranfield University (Glasshouse facility) to establish the long term role in terms of nutrient provision and the impact on soil chemical properties as a result of STSE irrigation on soils amended with compost. Pots were sown with perennial ryegrass (*Lolium perenne*) and soils used in the pot experiment were the same soils as used in the incubation experiment. In the first year, soils were sampled after each and every ryegrass cut and at the start and end of the second year. Plant and soil samples were analysed for N and chemical and physical soil properties respectively. This study was also designed to complement the results from the lysimeter study.

Lysimeter study: The lysimeter experiment was set up at Silsoe farm (Cranfield University) to determine the impact of compost and STSE nutrient integration on nutrient accumulation, leaching and N uptake. Leachate was collected periodically and analysed for N and P. Soils were analysed for P and N at the start and end of the experiment. This study augmented the results obtained from the pot (glasshouse) study but focussed on potential impact to the environment through potential leaching (if any).

Figure 1-1 summarises the methodological approach that was followed in the course of the research. It also shows the linkages between the individual experimental studies and the objectives of the research project.

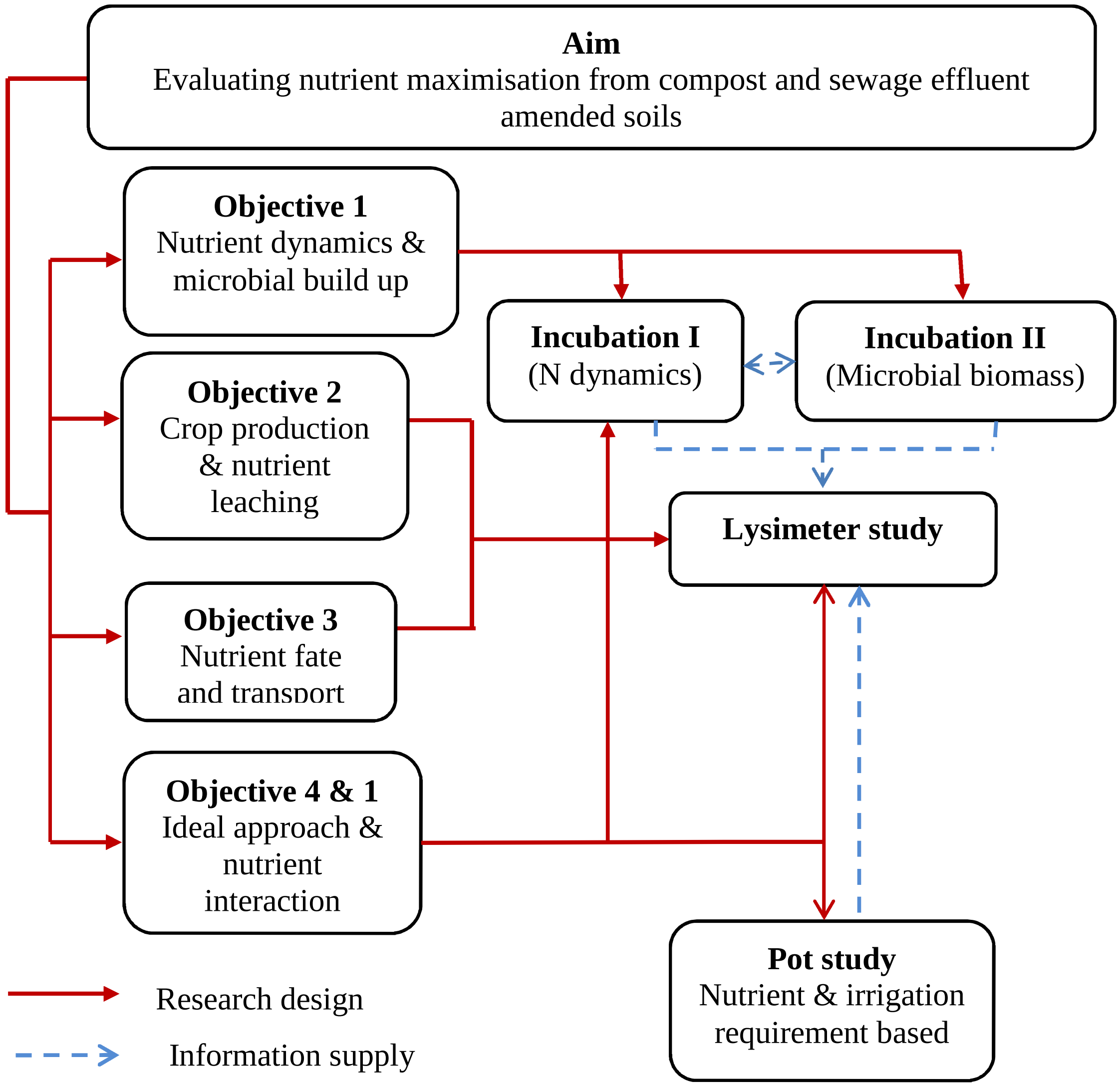


Figure 1-1 Methodological framework summarising the research approach and the linkage between experiments and objectives.

In the experiments, the main factors under consideration were the combinations of compost and STSE, soil type and N application rates. In all the experiments, two soil types were considered due to their distinct nature; clay loam and sandy loam. **Table 1-1** summarise the variables that were tested under each experiment and the underlying experimental factors.

Table 1-1 Summary of the variables tested and the experimental factors under consideration.

Experimental unit	Variables tested	Experimental factors
Incubation I	• N mineralisation • N dynamics • N kinetics	• Soil type • N application rates • Compost-STSE N combinations
Incubation II	• Microbial biomass C • Microbial biomass N	• Soil type • N application rates • Compost-STSE N combinations
Pot study	• Dry matter (DM) production • N uptake • N plant • Heavy metals - soil • Soil properties	• Soil type • N application rates • Compost-STSE N combinations
Lysimeter study	• N & P leaching • Soil properties • Dry matter production • TN_{plant} & N_{uptake} • P_{plant} & P_{uptake} • Heavy metals	• Compost-STSE N combinations • Soil type

Repeated ANOVA (General Linear Models) in Statistica 9.0 was conducted on the data to determine significant difference of means. Significantly different levels of treatments were identified using least significant differences at probability of 0.05 (Fishers LSD).

1.4 Thesis structure

Following the introduction presented in this chapter (**Chapter 1**), **Chapter 2** reviews the available information in the literature about composting, its usage in agriculture and wastewater recycling for crop production. It discusses the shortfalls and the knowledge gaps associated with application of either compost amendments or STSE alone in agriculture and the need for nutrient integration. **Chapter 3, 4** and **5** details the incubation, pot (glasshouse) and lysimeter experiments respectively.

In each chapter, the associated methodology and major findings are presented respectively. **Chapter 6** integrates the findings of Chapters **3, 4** and **5** and discusses practical implications of combining compost and sewage effluent. **Chapter 7** reports the overall conclusions of the research and provides ideas for future research.

2 LITERATURE REVIEW

2.1 Introduction

This chapter reviews existing available knowledge on compost recycling to agricultural land, utilisation of recycled water (STSE) and integrated nutrient application in agriculture. This literature review gives a detailed summary of current knowledge regarding the application of STSE and compost to agricultural soil while focusing on benefits and shortfalls of these nutrient sources. A review of knowledge on efforts to improve on the shortfalls of organic amendments has also been outlined with emphasis on integrated organic and inorganic fertiliser nutrient supply.

2.2 Agricultural utilisation of compost

Low-input agriculture has emerged as an important consideration as its popularity is motivated and supported by growing evidence of environmental and health risks from agrochemicals and the price escalation of inorganic fertilisers associated with conventional agriculture. Low input agriculture entails reduced dependency on inorganic fertilisers while relying on organic amendments. The rapid increase in population in developing countries coupled with increase in selling prices of inorganic fertilisers will affect usage of chemical fertilisers by smallholder farmers in these countries. Developing countries are the least users of inorganic fertilisers in the world. For example, according to FAO (2008), though nitrogen (N) consumption is forecasted to grow at 2.9%, and phosphate and potash by 1% and 2% respectively in 2011/12, the overall consumption is still marginal at less than 3% of the world consumption.

It is anticipated that between 50 and 70 million people will be added annually to the world population until the mid-2030s and almost all of this increase is expected to take place in developing countries especially the group of 50 least developed countries (FAO, 2008). Apparently this is also a region worst hit by rapid decline in soil fertility due to the scarcity of suitable agricultural land that has resulted in continuous cultivation and has exerted considerable pressure on land resources (Nalivata, 2007). More food and fibre will be required to feed and clothe this additional population. All these factors suggest that the adoption of sustainable low-input agriculture is essential mostly in low income countries, to increase the per capita global agriculture value that has not seen sustained increase in the last four decades (Wik et al., 2008).

The art and science of making compost for use to provide plant nutrients has been around for centuries. In most parts of the world, composting disappeared in the twentieth century as the use of chemical fertilisers increased, particularly following World War II (Epstein, 1997). Composting made a comeback towards the end of the last century due to economic and environmental concerns chemical agriculture raises and waste generation.

In some countries, the desire to compost waste has been hastened by the rapid rate at which available landfills are becoming full. In this case, deliberate policies have been put in place to prohibit yard waste from entering landfills and to recycle glass, metal, plastic and paper items. In the European Union, the Landfill Directive (CEC, 1999) is the main driver for the management of biodegradable waste. The implementation of the Landfill Directive sets demanding targets to reduce the amount of biodegradable municipal waste landfilled. In particular, the biodegradable municipal waste landfilled should be reduced to 75% of that produced in 1995 by 2006, to 50% by 2009, and to 35% by 2016 (Kokkora, 2008).

While policies help to regulate and monitor the quality of compost produced for agriculture in developed countries, the same is lacking in most developing countries. The inexpensive approach used to produce compost in most developing countries does not produce beneficial agricultural results and often ends up as a poor investment. However, addition of organic amendments improves the organic matter status of the soil. The application of organic wastes to soil has been demonstrated as an effective environmental and agricultural practice for maintaining soil organic matter (Tejada and Gonzalez, 2006), reclaiming degraded soils and supplying plant nutrients. There is a close relationship between the nutrient status of the soil and the organic matter content. As observed by Goyal (1999), application of organic amendments to soil improves both soil organic carbon (C) and total N. Soil organic C and N contents provide a measure of soil organic matter status. It has been shown by Chen et al., (2005) that in addition to supplying nutrients from mineralisation of organic matter, the advantages of higher availability of nutrients with soils of higher organic matter contents are multiple.

The fibrous portion of organic matter plays an important role in improving soil physical properties: promotes soil aggregation and improves permeability and aeration of clayey soils. Its high moisture-absorbing power and high carbon for growth of microbial mycelia help in the granulation of sandy soils to improve nutrient and water holding capacity (Indira et al., 2010). Organic matter accounts for at least half the cation exchange capacity (CEC) of soils. Thus, it is very important in retaining nutrients and increasing the buffering capacity of soils, enabling crops to better cope with such stresses as soil acidity and nutrient excess (Chen and Bejosano-Gloria, 2005). Organic matter provides food to a majority of microbes in the soil such that survival of the microbial population depends on availability of soil organic carbon and energy from the organic matter (Odlare et al., 2008). Decomposition of organic matter results in either mineralisation of nutrients and/or immobilisation from the humic substrates produced.

Composting is a useful method of producing a stabilised product that can be stored or spread with little odor or fly-breeding potential (Eghball, 2002). Manure for example, can be converted to compost by adding bulking agents to increase porosity during composting (Huang et al., 2001). Manure when mixed with nutrient-rich materials results in improved composting efficiency and compost quality (Leconte et al., 2009). Though application of compost leads to build up of soil organic matter, it may cause nutrient loading to surface and groundwater resources as a result in NO_3^- - N leaching (Basso and Ritchie, 2005).

Compost utilisation is on the increase worldwide. For example, in a report on the structure of the UK organics processing/recycling sector and the markets for its outputs by Gilbert et al., (2011), it showed that the estimated total organic waste inputs for composting across the UK have increased by 8.6% between the 2008/09 and 2009 reporting periods. A comparison of the quantities of wastes recycled in UK with previous years is shown in **Figure 2-1.** The amount of organic waste composted in the UK in 2004-05 was estimated at 2.67 million tonnes, increasing at a rate of about 35% and 145% from the amount composted in 2003-04 and 2000-01 respectively (Kokkora, 2008). The demand for nitrogen fertilisers will increase significantly in response to agricultural development, mainly in the developing world (Brentnall, 2008) and phosphorus is a scarce resource (Steén, 1998). These two scenarios will impact significantly on future trends of compost production and its usage.

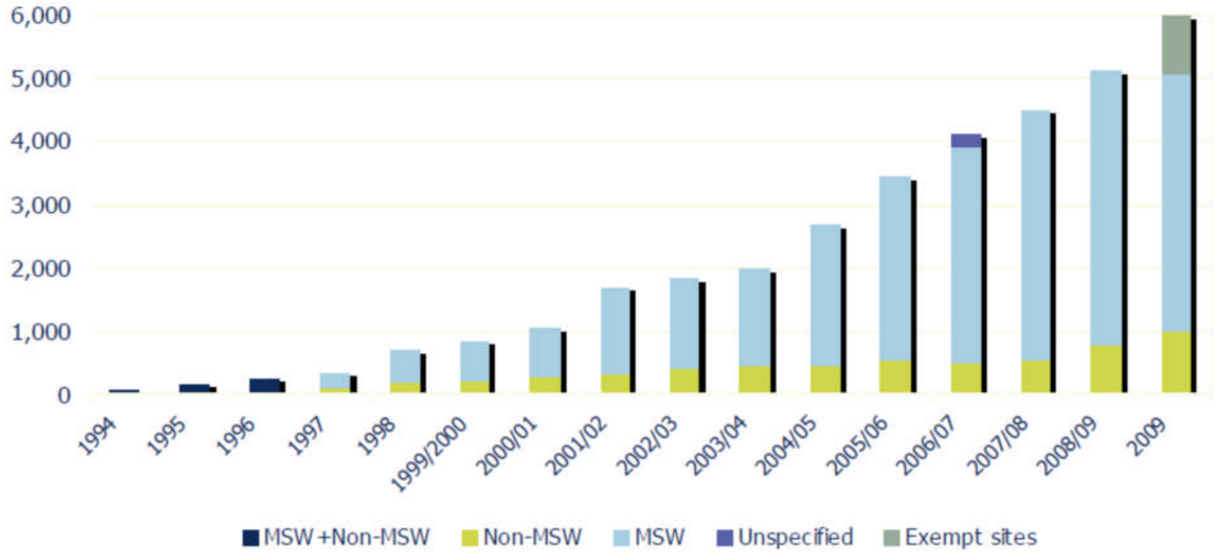


Figure 2-1 Growth in organic waste recycling in the UK (Source; Gilbert et al., (2011)). MSW stands for municipal solid waste.

2.3 Composting concepts

Composting is the biological degradation of organic substrates whose major products are CO_2, water and energy under high temperatures (Kaboré et al., 2009). According to Dalzell (1987), the decomposition of organic waste materials takes place in warm, moist and aerated environments. Compost can be made from green waste (Parkinson et al., 1999), agricultural wastes (Nalivata, 2007), bio-solids (Kokkora, 2008), crushed cotton gin (Tejada and Gonzalez, 2006) and food-waste (Shimozono et al., 2008), just to mention a few.

Compost has numerous advantages to both manure and inorganic fertilisers. In turf grass, compost plays a significant role in replacing degraded soil physical properties and is also capable of reducing the frequent use of pesticides by suppressing several diseases (Shimozono et al., 2008). Dalzell et al., (1987) outlined a number of advantages of using compost, ranging from improving soil fertility, improving cropping techniques to getting rid of excess wastes. In a maize production system, by analysing cumulative CO_2 accumulation or soil respiration, Eriksen et al., (1999) concluded that compost increased microbial population in soils that were amended with municipal solid waste compost. It was found that after a 55-day incubation experiment, the maximum cumulative CO_2-C of 3600 mg 100 g soil^{-1} when 126 Mg ha^{-1} of municipal solid waste compost was applied as compared with 400 mg 100 g soil^{-1} in the control treatment without any amendment. An increase in CO_2 accumulation is a good indicator of microbial population presence in the soil.

The composting process is controlled by various factors. These factors are significant as they affect the quality of the end product. According to Epstein (1997) and Dalzell et al., (1987), moisture, temperature, agitation, nutrients and pH are essential in the composting process as they create a conducive environment for microorganisms to work on the organic matter.

2.3.1 Moisture

Moisture is required by the microorganisms working on the compost. At moisture content below 30% on a fresh weight basis, the biological reactions in a compost heap slows down considerably (Dalzell et al., 1987). Epstein (1997) stipulated that at moisture content of about 40%, microbial activities begin to slow down. Too much moisture saturates the compost heap leaving all pores filled with water. At moisture content of above 60%, oxygen availability is restricted in the compost mass (Epstein, 1997). In composting, moisture or water is produced by the microorganisms and is lost by evaporation. According to Dalzell et al., (1987), the optimum moisture content of the ingredients for composting is 50 – 60%, additional water is added when there is a reduction in moisture content in a compost heap.

2.3.2 Temperature

Temperature is a significant factor in composting though it is also a product of the composting process. The process of decomposition of organic matter produces heat hence the rise in temperature. The rise in temperature affects the micro-organisms as population change from mesophilic to thermophilic organisms (Dalzell et al., 1987). Higher temperatures (>55˚C) are necessary to kill pathogenic microorganisms for example, total faecal coliforms and specifically *Escherichia coli*, faecal *Streptococci*, *Staphylococci*, *Salmonella*, and *Shigella* (Hargreaves et al., 2008). However, if the temperatures exceed 63˚C, microbial activity declines rapidly as the optimum for various thermophiles is surpassed, with activity approaching low values at 72˚C (Bernal et al., 2009).

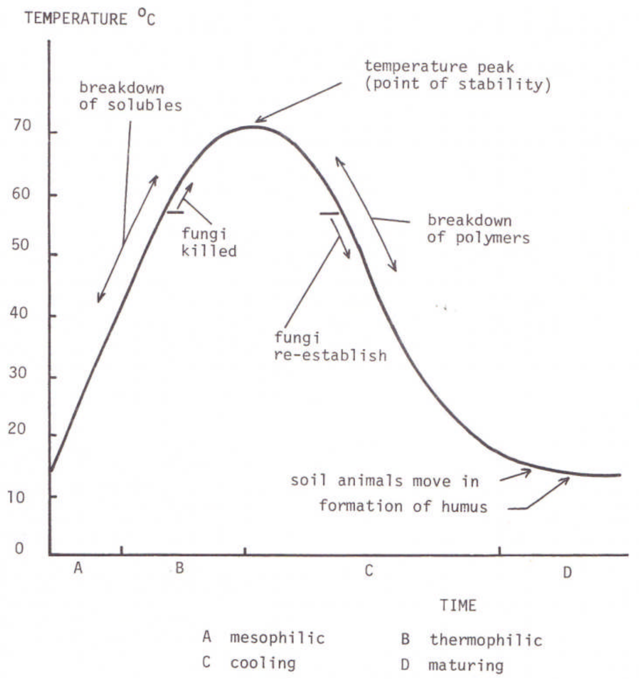


Figure 2-2 Temperature variations in compost mass (Source: Dalzell et al., (1987)**)**

A compost mass undergoes various temperature changes (**Figure 2-2)**. Bernal et al., (1998b) cited four important stages in composting that are strongly linked to the temperature evolution of **Figure 2-2**: (1) the initial mesophilic stage, when raw materials have not yet undergone decomposition; (2) the thermophilic phase, when the material reaches its maximum temperature (>40˚C) and is degraded rapidly; (3) end of the bio-oxidative phase which is marked by a fall in temperature (cooling); (4) maturation phase.

2.3.3 Nutrients

Availability of nutrients is essential for the growth of micro-organisms responsible for the decomposition of organic matter. Both micro and macro nutrients are needed for propagation of microbial organisms, however due to limited availability of data on the micro-nutrients (Epstein, 1997) attention is paid to N and C. N is used for developing cell proteins for the microbes (Dalzell et al., 1987) and can limit microbial activity and the rate of decomposition of organic matter. According to Troech and Thompson (1993), N is required in fixed ratio's to the amount of C going into the body of the micro-organisms, therefore the higher the C/N ratio, the longer the time taken for micro-organisms to decompose organic matter (Epstein, 1997; Dalzell et al., 1987; Troeh and Thompson, 1993). Higher C/N ratio implies that more C is available in the soil for microbial energy as compared to N that is needed for cell development. At a C/N ratio exceeding 50:1 the composting process slows down because of rapid cell growth and depletion of available N, resulting in reduced cellular growth (Epstein, 1997). But low C/N values under alkaline conditions can lead to NH_3 volatilisation (Tam and Tiquia, 1999). Composting materials with C/N < 10 result into nutrient loss through volatilisation (Tam and Tiquia, 1999). Tam and Tiquia (1999) concluded in their research that C/N ratio is the most critical factor affecting the changes in total and organic N concentration during co-composting of spent pig manure, sawdust litter and sludge. Total N content is an important compost property, as the amount of compost application is often restricted or regulated according to the amount of total N applied to the soil (Kokkora et al., 2009).

The concentration of total N usually increases during composting when volatile solids (organic matter) loss is greater than the loss of NH_3 (Bernal et al., 1998a). However, volatilisation is one of the common losses of N that often results in the decrease of total N concentration during composting as was observed by Nalivata (2007) and Tumuhairwe et al., (2009). As observed by Eghball et al., (1997) during composting, depending on the type of composting system, waste stream (e.g. lignin content) and composting conditions (temperature and moisture), N loss ranges from 19 to 42% while C loss ranged from 46 to 62%. The majority of C loss is from carbohydrates, hemi-cellulose and cellulose as they constitute the majority of plant C and especially during active decomposition by thermophilic microorganisms (Kuo et al., 2009). The end result is a decline in C/N ratio in the compost mix until a steady state condition is reached.

Composting methods also influence the dynamics of nutrients and the final nutrient content of compost. Tumuhairwe et al., (2009) compared four low composting technologies (open pit, covered pit, above ground open and above ground covered composting methods) for market wastes. They concluded that despite all methods producing mature compost in 63 days, compost handling e.g. turning frequency, affected N dynamics. Turning during composting hasten ammonia volatilisation (Ogunwande et al., 2008) and open weather conditions (in case of uncovered composts) expose compost to various factors including rainfall that enhances leaching and result into increased N losses.

2.3.4 pH

The alkalinity or acidity of the compost mass affects the growth response of the micro-organisms (Epstein, 1997). When the mass turns slightly alkaline after a few days of composting, proteins are attacked by microbes and ammonia is produced (Dalzell et al., 1987), however if the pH continues to increase, N is lost as ammonia. According to Guerra-Rodríguez et al., (2003), it is possible to compost in the pH range of 3 to 11 but best results are obtained between pH 5 and 9.

2.4 Composting methods

Broadly, composting methods are divided into four groups: passive composting, windrows, aerated piles, vermicomposting and a group of methods collectively known as in-vessel composting. Selection of which method to use depends on a number of factors e.g. the size of the manure to be composted, availability of resources, space and availability of labour.

2.4.1 Passive composting

In passive composting, feed stocks are placed in a pile where air circulation is natural, making the composting process slow and has a greater potential for odour problems as little or no air passes through the pile. This leaves anaerobic microorganisms to dominate the decomposition process in the pile. Effectiveness of passive composting is attained with the incorporation of beddings to the piles (Rynk, 1992). The beddings improve the porosity of the piles thereby improving the circulation of air in the piles and allowing some parts of the pile to undergo aerobic decomposition. Smallholder farmers have modified the passive composting process to get the most from it. In Malawi for example (**Figure 2-3**), piles insulated with mud made on a constructed base with a stick inserted in the middle of the pile to create an air passage from the bottom to the top of the heap are common with smallholder farmers (Nalivata, 2007). The system is cheap though anaerobic conditions can develop if not properly controlled (Bernal, 2008).

Figure 2-3 Modified passive composting (Chimatu) in Malawi

2.4.2 Windrows composting

Windrow composting involves piling of waste in long narrow rows or piles (**Figure 2-4**). It is suitable for large quantities of waste and is undertaken for on commercial purposes. The piles are mixed/turned regularly by specialised equipment to improve porosity and oxygen content, mix in or remove moisture and redistribute cooler and hotter portions of the pile. Turning fluffs up the windrow and restores the pore spaces eliminated by decomposition and settling thereby improving air exchange (Rynk, 1992).

The high land requirement of windrow composting is a significant drawback of the method. The leachate, which is released during the composting process, can contaminate local ground-water and surface-water supplies and thus, should be collected and treated (Kokkora, 2008). Because of the required management of the windrows and the capital intensiveness of equipment required for turning the windrows, windrow composting is not common with smallholder farmers.

Figure 2-4 Windrows composting (Source: Mid-Atlantic Composting Directory, http://pubs.ext.vt.edu/452/452-230/452-230.html)

2.4.3 Aerated static piles

In static aerated piles, a blower is used to supply air to the composting materials through pipes (Rynk, 1992). In this case once the piles have been formed, no turning or agitation of the materials is required. Due to the associated costs of the system, smallholder farmers in developing countries, aerate the piles by manually turning the materials in the compost regularly to promote circulation of air and uniform decomposition of the feedstock within the piles.

2.4.4 In-vessel composting

In-vessel composting refers to composting methods in which the composted materials are confined in a building, container or vessels. In these structures, air is forced into the material and mechanical turning techniques are used to speed up the composting. Many methods combine techniques from windrows and aerated piles methods in an attempt to overcome deficiencies and exploit the attributes of each method (Rynk, 1992).

2.4.5 Vermicomposting

Vermicomposting methods involve the degradation of composted materials by microorganisms and worms (BSI, 2005). Unlike thermophilic composting that depends on increasing temperature, it can be successfully managed on large or small scale hence it is widely practiced in kitchens, homemade bins or crates on small scale. The greatest challenge of vermicomposting is to ensure that the feedstock do not attain temperatures high enough to begin thermophilic process of decomposition, as this would kill worms and microorganisms (McClintock, 2004). According to Misra et al., (2003), vermicomposting method results in high quality compost.

2.5 Nutrient availability from compost amended soils

2.5.1 Nitrogen

Compost application enhances nutrient availability and organic matter status of the soil (Parkinson et al., 1999). Application of compost for crop production increases yield through the contribution of organic matter to the soil. N derived from compost amendments mainly acts through the soil organic N pool (Gutser et al., 2005) as the mineral N content of compost is low due to nutrient losses during composting (Hao et al., 2004; Tiquia et al., 2002). In the soil organic N pool, compost N is organically bound because of the slow release characteristic which is due to the nutrient stability in compost; availability of N for crop utilisation is low in the year of application depending on the C/N ratio. In terms of soils, C/N ratio of 15 is a critical limit separating soil groups with higher or lowers N release (Springob and Kirchmann, 2003). **Figure 2-5** illustrates the fate of the compost-N in relation to the soil N pool.

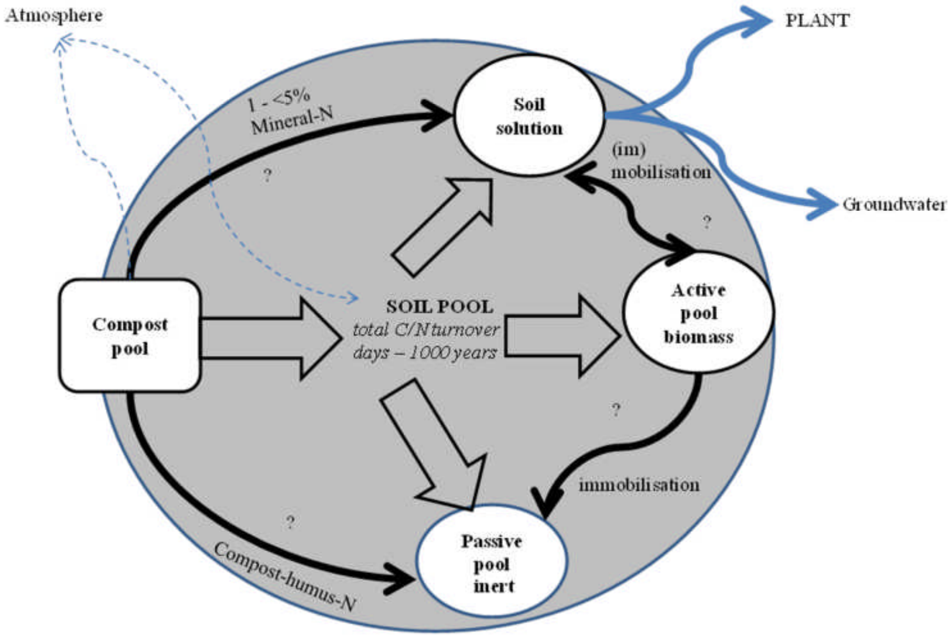


Figure 2-5 Fate of compost–N in the soil organic matter (SOM) – N pool (Source: Amlinger et al., (2003)**).**

According to Amlinger et al., (2003) availability of compost-N depends on the C/N ratio of the original feedstock and that of the final compost, composting conditions, decomposition or stabilisation rates, climate, soil, duration of the composting and the post-treatment of compost. Wider C/N ratios cause soil N to be immobilised while narrower ratios permit N mineralisation to occur as the organic matter decomposes (Troeh and Thompson, 1993). According to Tisdale et al., (1990), organic materials with a C/N ratio of less than 20, usually releases mineral N early in the decomposition process as compared to those with C/N between 20 and 30. Organic materials with C/N greater than 30 will result in immobilisation during the initial decomposition process.

Mineralisation of N involves ammonification (**Equation 2.1**) and nitrification processes. During the ammonification process, organic N is transformed to NH_4^+ and NH_3.

$$Organic\ \text{-}\ N \rightarrow NH_4^+ \longleftrightarrow NH_3$$ **Equation 2-1**

NH_4^+-N is further converted to NO_3^- through nitrification by *Nitrosomonas* sp. and *Nitrobacter* sp. through a two-stage oxidation process (**Equation 2.2 & 2.3**).

$$2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+ + energy$$ **Equation 2-2**

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- + energy$$ **Equation 2-3**

The C/N ratio of the final compost is a major decisive factor for the availability of N to crops. Initial immobilisation of N is likely to occur when compost with higher C/N ratio is applied in the soil. Soil microbes firstly use the N from surrounding areas to build their biomass and give off the excess carbon through respiration. According to Hue et al., (1999) this process continues until the C/N of the amended soil is in equilibrium with that of the microbes. Initial immobilisation of N may not necessarily be undesirable as it can reduce NO_3^--N leaching and potential ground water pollution (Hue and Sobieszczyk, 1999).

Although various site-specific factors (e.g. compost maturity, composting conditions, climate, soil properties and soil management) may affect N-dynamics in compost amended soils, it can generally be assumed that the availability of N to plants is low since the majority of (>90%) of total compost N is bound to the organic N-pool (Amlinger et al., 2003). The gradual release of N from the soil organic-N pool and the low mineralisation rates affect crop yield in the first year of application mainly when compost alone is applied as a source of crop nutrients. The end result is that high yields are often associated with increased compost application rates, for example after applying 50 t ha^{-1} greenwaste compost annually for three years, Parkinson et al., (1999) found additional fresh weight yield response of maize of 30.9 t ha^{-1}, representing 75% increase relative to fields that were not amended with greenwaste compost. Similarly, higher mean yields of 9.9 and 10.9 Mg ha^{-1} of corn were obtained in a five year experiment in treatments with high compost application rates of 33.6 and 44.8 Mg ha^{-1} respectively (Smiciklas et al., 2008).

Amlinger et al., (2003) reported N mineralisation rates of between 5-15% (incubation experiment) and 3-5% (21-year field experiment) in the first year of compost application while Gutser et al., (2005) and Passoni and Bonn (2009) reported N mineralisation of between 0 – 20% and 35 – 40% respectively. However, these results are not comparable as the experiments were undertaken on different soils, soil management and with different compost types. Amlinger et al., (2003) determined the mineralisation rate from biowaste and yard waste compost amended soils while Passoni and Bonn (2009) made their determination from food and municipal waste compost, green cutting residues and organic fraction of municipal waste solid residue compost and municipal sludge and green cuttings residues compost. In general, mineralisation rates of compost are low rendering compost less efficient as a nutrient source.

The residual compost-N for soils amended with biowaste and yard waste compost in a field experiment is mineralised at rates of about 3-5% in the second year and 1.5-2% in each of the following years (Amlinger et al., 2003). Depending on the degree of maturity, total N concentration in compost, type of compost and soil type, almost 20% of total N can be plant available in the soil after 70 days of incubation (Bernal et al., 1998c).

Evaluation of effects of application of organic amendments on nitrate leaching has often resulted into discordant findings. Mallory and Griffin (2007) in a 13-year cropping systems experiment found that, despite similar NH_4^+-N inputs and rates of NH_4^+-N consumption for manure and fertiliser N treatments, NO_3^--N accumulation was slower in the manure treatment. This was attributed by Diacono and Montemurro (2010) to the slow availability of N from manure as compared to fertilisers. In contrast, Basso and Ritchie (2005), in a 6-year maize-alfalfa rotation, observed the highest amount of NO_3^--N leaching of 681 kg ha^{-1} in the manure treatment, followed by compost (390 kg ha^{-1}), inorganic N (348 kg ha^{-1}) and control treatment (311 kg ha^{-1}). N from organic materials can be mineralised in the soil at a time when no crop uptake is taking place, leading to a greater leaching potential (Kirchmann and Bergstrom, 2001). As suggested by Diacono and Montemurro (2010), attention needs to be given to environmental protection when organic amendments are used in agricultural systems.

2.5.2 Phosphorus

Phosphorus is one of the growth-limiting macro nutrients whose occurrence is not as abundant as other macro nutrients. Soil P is classified as organic or inorganic depending on the nature of the compounds in which it occurs or to which it is bound. The inorganic fraction of P occurs in numerous combinations with iron, aluminium, calcium, fluorine and other numerous elements (**Figure 2-6**). According to Tisdale et al., (1990), inorganic P content of soil is frequently higher than that of organic P except in soils that are predominantly organic. Because of the accumulation of organic matter in the upper part of the soil profile, organic P is usually higher. Most naturally occurring organic forms of P are esters of orthophosphoric acid and numerous mono- and diesters (Tisdale et al., 1990). According to Troeh and Thompson (1993), identifiable organic P compounds in the soil include inositol phosphates, nucleic acids and phospholipids.

P is absorbed largely as primary and secondary orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}), though according to Troeh and Thompson (1993), $H_2PO_4^-$ is the common form of P absorbed by plants. At pH less than 7.2, $H_2PO_4^-$ is the main form and it is predominant in many agricultural soils (Tisdale et al., 1990) while the importance of HPO_4^{2-} ion increases at high pH because it becomes the dominant ion in soil solution above pH 7.2. pH controls the solubility of P ions thereby influencing not only the kind of P ions present but also the concentration of the precipitating ions. Solubility of P ions is also governed by temperature and the moisture content of the soil.

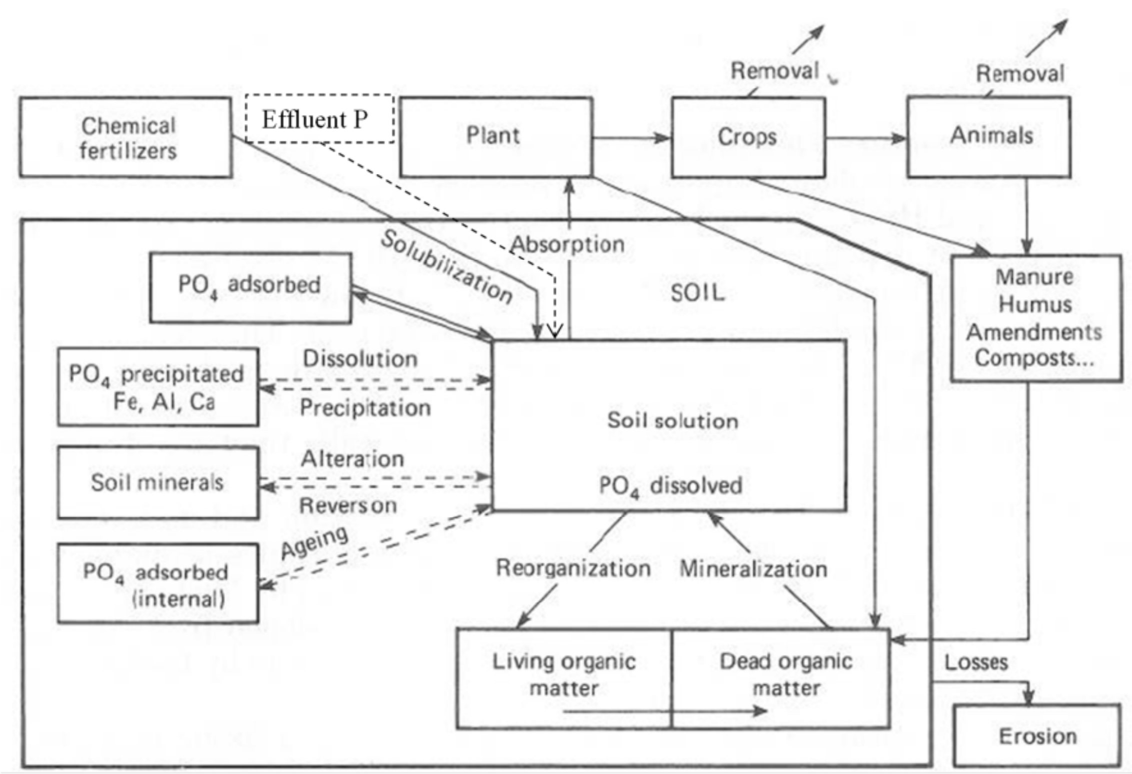


Figure 2-6 Phosphorous dynamics showing the contribution of effluent P and compost P in the soil (Adapted from Tisdale et al., (1990)**).**

In a compost and STSE nutrient integration, effluent – P will act directly through the soil solution while the compost P will undergo mineralisation and be dissolved in soil solution before absorption by plant roots (**Figure 2-6)**. Once in soil solution, effluent – P can be immediately accessible and available for plant uptake (**Figure 2-7**).

In organic amendments, P is held by covalent bonds and as such cannot ionise to become readily available in soil solution (Tisdale et al., 1990). P is usually surrounded by oxygen and attached to the rest of the molecule by a carbon-oxygen-phosphorous bond sequence. Through decomposition, the bond is broken and P is mineralised into forms that are available for plant uptake and growth (**Figure 2-6**). Organic P from compost mostly acts through the strongly-bonded, absorbed P pools or the very strongly bonded or inaccessible pool (**Figure 2-7**) hence limited availability to plant roots in the soil.

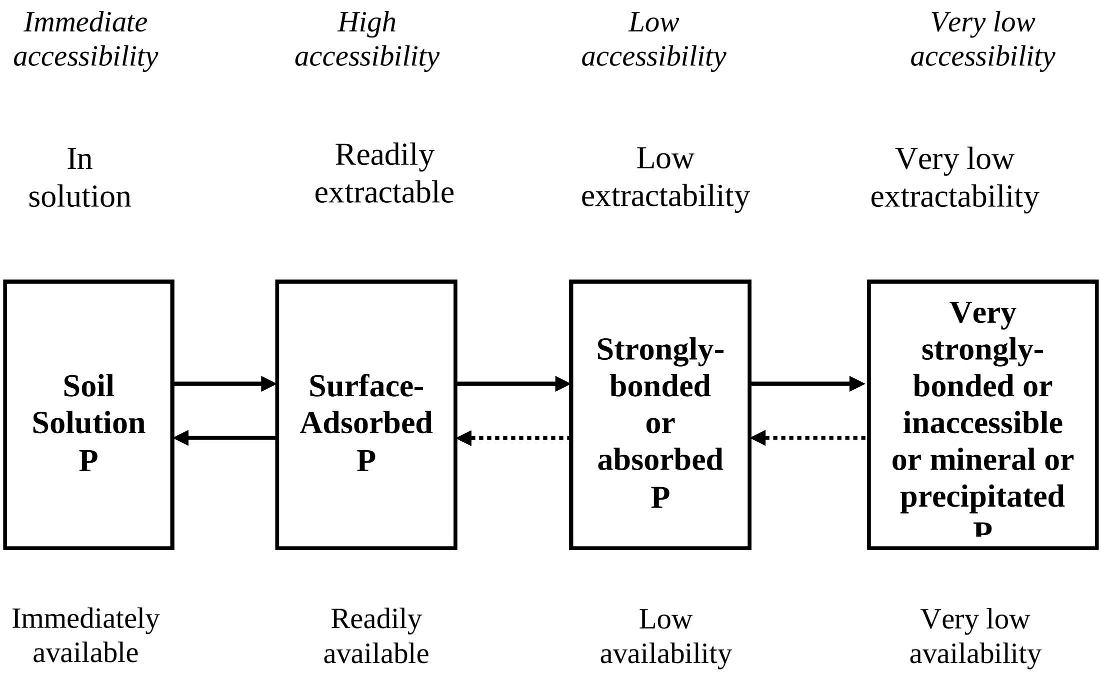


Figure 2-7 Conceptual diagram for the forms of inorganic phosphorous in the soil categorised in terms of accessibility, extractability and plant availability (Source: Syers et al., (2008)).

The proportion of P in compost is often very low and so is the contribution of compost to soil P but the organic matter provided by the compost plays a very significant role in the availability of phosphorous. The presence of organic matter in the soil controls the dynamics of phosphorous in soils. The rate of plant uptake of P is influenced by the availability of N (Troeh and Thompson, 1993; Palm et al., 1997) that can have its origin from organic amendments. Organic amendments reduce P sorption and organic complexation of cations (e.g. Al, Fe and Mg) that limit P solubility (Hue and Sobieszczyk, 1999). According to Troeh and Thompson (1993), complexing leaves the cations (Al, Fe and Mg) in solution to precipitate insoluble P compounds thereby preventing the leaching of phosphorous (**Figure 2-7**). Decomposing organic matter releases acids that increase solubility of calcium sulphate thereby increasing the amount of available P (Troeh and Thompson, 1993). Smiciklas et al., (2008) observed accumulation of P in the soil when food waste and ground newsprint compost was applied to the soil. However, Smiciklas et al., (2008) in their experiment used very high compost application rates of up to 34 Mg ha^{-1}.

2.5.3 Role of carbon and microbes in nitrogen availability

Adequate inputs of organic matter are vital for maintaining the fertility of arable soils and retaining atmospheric carbon dioxide (CO_2) in the soil organic matter pools thereby improving organic carbon content in the soil (Vleeshouwers and Verghagen, 2002). Soil fertility is the quality of soil that enables it to provide essential chemical elements in quantities and proportions for the growth of specified plants (Brady and Weil, 2008). Soil fertility depends on the chemical, physical and biological characteristics of soil. Organic carbon is utilised for building body tissue and as an energy source by decomposer microorganisms and its fate is to be assimilated into their tissues, released as metabolic products or respired as CO_2 (Diacono and Montemurro, 2010). Application of organic amendments increases soil organic carbon. Over a 5-year period, Habteselassie et al., (2006) found that soil C pool was enhanced by 115% in dairy-waste treated soil while Montemurro et al., (2006) observed an increase of total organic carbon of 24 and 43% after a 3-year soil amendment with municipal soil waste compost and olive pomace compost respectively. Similarly, Eghball (2002) reported increased soil carbon after long term application of compost and/or manure. Liu and Haynes (2011) reported no increase of organic carbon after long-term treated dairy effluent irrigation but concluded that the additional inputs of soluble carbon in treated effluent are approximately balanced by either losses or microbial usage of carbon of a similar magnitude hence no build-up of soil carbon stocks.

Microorganisms play a significant role in decomposing organic matter and nutrient cycling. Through decomposition of organic matter, organic N and P are converted into inorganic forms usable by plants. Assimilation of decomposing organic matter is governed by the ratio of C to N in the microbial biomass. According to Tisdale et al., (1990), the C/N ratio of organic materials added to soils will have pronounced effect on positive (mineralisation) and negative (immobilisation) N release such that when organic materials with C/N ratio wider than 30:1 are added to soil there is immobilisation of soil N. Diacono and Montemurro (2010) reported that the amount of N required by microorganisms is 20 times smaller than that of C. If the concentration of easily decomposable C is low with a larger quantity of N in respect to that required by microbial biomass, there will be net N mineralisation.

Microbial biomass growth and function are related to substrate C input. Amending the soil with organic materials always induces an increase in soil microbial biomass (Zhang et al., 2005). Addition of soluble organic materials through effluent stimulates microbial activity (Liu and Haynes, 2011). After four years of amending soils with compost and manure, Ginting et al., (2003) reported a 20 to 40% increase of soil microbial biomass. The quantity and quality of organic materials applied to soils are the major factors controlling the abundance of different groups and the activity of microorganisms involved in nutrient cycling (Diacono and Montemurro, 2010). Monaco et al., (2008) concluded that microbial respiration is affected by the nature of the C input and it is lower in organic materials with partially humified carbon. The quality of carbon is particularly important because it constrains the supply of energy for enzyme production and growth (Fontaine et al., 2003).

To better understand the mechanism through which C is stored or lost, it has been separated into three pools; a labile or actively cycling pool (<5% with turnover times ranging from hours to months), a slow pool (20-40% C with decadal turn over times) and a stable or passive, recalcitrant pool with varying residence times (60-70% C with a turnover ranging from centuries to millennia) (Sherrod et al., 2005; Lal et al., 2007; Parton and Rasmussen, 1994; Majumder et al., 2008). According to Majumder et al., (2008), the labile/actively cycling carbon pool is important from the point of view of crop production. It fuels the soil food web and greatly influences nutrient cycling for maintaining soil quality and its productivity (Janzen, 1988).

C compounds play significant roles in its availability in the soil. Humic substances contain a variety of functional groups, including COOH, phenolic OH, alcoholic OH, quinone, hydroxyquinone, lactone, and ether (Stevenson, 1994). These humic substances play an important role in the physicochemical properties of soil through (1) a positive effect on the structure of soil (Stevenson, 1994); (2) a source of nutrients and trace metals and regulation of the supply of nutrients from other sources in the soil (Torrecillas et al., 2013); and (3) a positive effect on the activity of micro floral and micro faunal organisms (Burns et al., 1986).

2.5.4 Modelling N availability

Mineralisation of organic N is of major importance and a prerequisite of the N supply to plants (Appel and Mengel, 1993; Cordovil et al., 2005). The rate of N mineralisation is controlled by the environment, chemical composition of the organic material and the soil microbial pool (Cordovil et al., 2005). Fitting kinetic models to incubation data has been used to predict and estimate potentially mineralisable N.

Modelling N mineralisation kinetics in soil usually involves the prediction of an active fraction of the total or organic N and a rate constant to predict the rate of mineralisation (Benbi and Richter, 2002). Stanford and Smith (1972) defined soil N mineralisation potential as the quantity of soil organic N susceptible to mineralisation at a rate of mineralisation (k). The first order kinetic model of Stanford and Smith (1972) assumes the existence of a single pool of soil organic matter. The Stanford and Smith (1972) model assumes that the pool containing the compounds that really contribute to the potentially mineralisable N is similar in most soils hence the one pool model. However, other authors consider the one pool model unsatisfactory (Deans et al., 1986; Matus and Rodriguez, 1994) hence multi-fraction approaches (Benbi and Richter, 2002). Nevertheless, the one pool model of Stanford and Smith (1972) has been used extensively to estimate the potentially mineralisable N in soils due to organic amendments (Cordovil et al., 2005; Benbi and Richter, 2002; Gil et al., 2011; Serna and Pomares, 1992).

2.6 Wastewater irrigation

2.6.1 Water recycling for agricultural production

Crop irrigation with effluent (treated or not) is a worldwide practise in drought affected as well as in humid regions as a source of nutrients and to meet crop water requirement for crop production (Khalil-Gardezi et al., 2009; Lado and Ben-Hur, 2009). Wastewater has been recycled in agriculture for centuries as a means of disposal in cities such as Berlin, London, Milan and Paris (Pedrero et al., 2010). In Pakistan, 26% of national vegetable production is irrigated with wastewater (Ensink et al., 2004) while in Ghana, informal irrigation involving diluted wastewater from rivers and streams occurs on an estimated 11,500 ha, an area larger than the reported extent of formal irrigation in the country (Keraita and Drechsel, 2004) as cited in (Pedrero et al., 2010). Water recycling is expected to reach 10 to 13% of water demand in the next few years in Australia and California (Lazarova and Bahri, 2005).

The rapid development of irrigation with STSE that has occurred in the last 20 years has to a large extent been stimulated by increasing water shortages and facilitated by new policies and regulations (Lazarova and Bahri, 2005). However, in some countries usage of recycled water is propelled by the need to supply both water and nutrients for plant growth. Despite extensive utilisation of recycled wastewater/effluent, shortfalls have been noted by various researchers as excessive inputs of some elements have adverse impact on plants. For example, the high total N of reclaimed water from secondary treatment makes it unfavourable for crop growth (Chiou, 2008). NO_3^--N content > 30 mg l^{-1} presents a severe restriction on wastewater usage in agriculture (**Table 2-1**). Excess vegetative growth, lodging, delayed maturity and reduced fruit quality are some of the consequences of excessive nutrient supply.

Wastewater irrigation also affects electrical conductivity of the soil. After a long term untreated domestic waste water irrigation (25-30 years), Simmons et al., (2010) reported a 50% increase in electrical conductivity in comparison to canal water irrigated soil. Xu et al., (2010) and Samaras et al., (2009) concluded that usage of reclaimed wastewater resulted in an increase in soil salinity. As salinity increases, the probability of certain soil and cropping problems increases as crops tolerate soil salinity differently. Over irrigation with effluent of medium salinity helps to leach salts to lower horizons but this practise in most cases has resulted in groundwater pollution from shallow aquifers. In specific situations, high soil salinity can affect water uptake by plants due to higher concentration in the soil solution of Na^+, Cl^- and HCO_3^- (Fonseca et al., 2007a; Bielorai et al., 1984).

In terms of yields, Fonseca et al., (2005a), in a pot-green house experiment reported that despite a slight increase in total N in the soil from 0.7 g kg^{-1} to 0.72 g kg^{-1} after irrigation with STSE, maize dry matter was not responsive. They found total dry matter yield of maize of about 20 g pot^{-1} in treatments receiving only secondary treated effluent as compared to 90 g pot^{-1} and 140 g pot^{-1} for treatments with a combination of STSE and mineral fertilisation, except N and STSE and complete mineral fertilisation respectively. Parson et al., (2010) also concluded that there is usually insufficient macronutrient content in STSE to meet plant nutritional requirements.

Chakrabarti (1995) reported lower wheat yields of 2.5 t ha^{-1} when irrigating with raw sewage without additional fertilisation as compared to 2.8 t ha^{-1}, with fertiliser fortified well-water irrigation. However, a considerable increase in yields (3.2 t ha^{-1}) was obtained as a result of a combination of chemical fertilisers and raw sewage. Chakrabarti (1995) therefore concluded that application of small quantities of inorganic fertilisers as a supplement to irrigation with raw sewage considerably improves yields. Similarly, Fonseca et al., (2005a) concluded that although effluent is considered a mineral enriched wastewater; it cannot be used as the only source of nutrients to plants.

Similarly, Ramirez-Fuentes et al., (2002), after assessing nutrient dynamics in soils amended with treated effluent since 1912 in an incubation experiment, concluded that despite increased concentration of organic C, total N and microbial biomass C and N (on average with 80 mg C kg^{-1} soil $year^{-1}$ and 14 mg N kg^{-1} soil $year^{-1}$ or a 1.4-fold and 3-fold increase, respectively), the nutrients could not maintain and sustain crop production. Hence they recommended additional application of N to maintain the same level of crop yields. This can reduce the quantity of effluent applied thereby reducing leaching of NO_3^- and N losses through denitrification by a controlled application of N either by inorganic fertiliser or organic fertilisers.

The stimulated microbial activity due to substrate inputs from STSE result in depletion of soil organic carbon. Though the effect has no direct influence on soil fertility of the rooting zone, the enhanced mineralisation of organic material results into greater amounts of CO_2 released from the soil which changes the C-balance and may therefore contribute to climate change (Jueschke et al., 2008).

Irrigation with effluent (treated or not) is considered an environmental hazard as heavy metals and nutrients can leach to ground water. Discharge of effluent, treated or untreated in natural water bodies such as rivers and lakes can cause problems such as eutrophication and algal blooms (Toze, 2006). Apart from that, excess NO_3^--N in potable water may lead to infant mortality resulting from a reduction of NO_3^- to NO_2^- by microorganisms in children's stomachs and in the rumen of animals (Fonseca et al., 2007b). The NO_2^-oxidises iron in the haemoglobin of red blood cells to form methemoglobin, which lacks the ability to carry sufficient oxygen to the individual body cells causing the infants to develop a blue coloration and respiratory problems known as methemoglobinemia, sometimes referred to as ''blue baby syndrome'' (Basso and Ritchie, 2005).

However, in the above study, by Chakrabarti (1995) he did not explore the effect of STSE used on the quantity of nutrients and heavy metals leached and accumulated into the soil However, in terms of heavy metals, accumulation of Cd, Pb and Zn in *Brassica oleracea var. Italica* (Broccoli) was observed when STSE was used for irrigation of the plant (Kalavrouziotis et al., 2008). Although the concentrations of heavy metals can be low in STSE from household origin, its application over the years has led to significant increases in some total heavy metal concentrations (Gwenzi and Munondo, 2008). In fact, inconsistent results have been reported indicating increasing, decreasing or no effect on soil heavy metal concentration as a result of crop irrigation with STSE. Mohammad and Mazahreh (2003) observed no effect on soil Cu (Copper), Zn (Zinc), cadmium (Cd), chromium (Cr), Nickel (Ni) and lead (Pb). Similar results were obtained by Johns and McConchie (1994) in a 2-year lysimeter experiment. Mapanda et al., (2005) reported concentration of heavy metals (Cu, Zn, Ni, Cd, Cr and Pb) content exceeding their maximum permitted limits after a long term irrigation (at least 10 years) with sewage effluent. Sewage irrigation for 20 years resulted into significant build-up of diethylenetriaminepentaacetic acid (DTPA) extractable Zn (208%), Cu (170%), Fe

(170%), Ni (63%) and Pb (29%) in sewage effluent-irrigated soils over adjacent tube-well water irrigated soils, whereas Mn was depleted by 31% (Rattan et al., 2005).

2.6.2 Brief review of sewage treatment process

Sewage treatment is a multistage process involving unit operations in which the removal of contaminants is brought about by chemical or biological reactions. The treatment process produces two products: (i) biosolids (sewage sludge) and (ii) effluent. Treated effluent is discharge in inland waters, estuaries and the sea whilst sewage sludge is spread in agricultural fields or in landfills.

Understanding the sewage treatment process is essential to understand the implications of the treatment options to the characteristics of the final product (in this case, effluent). Sewage treatment involves a variety of methods and processes. The trickling filter system is one of the processes through which sewage is treated (**Figure 2-8**). This review will focus on the trickling filter system. Sewage treatment at Cranfield Sewage Treatment Plant (source of the sewage effluent for the research study) uses the trickling filter system for sewage treatment.

The removal of carbonaceous BOD, the coagulation of non-settleable colloidal solids, and the stabilisation of organic matter are accomplished biologically using a variety of microorganisms, principally bacteria (Metcalf and Eddy, 1972).

In operation, wastewater is distributed evenly over the surface of the trickling filter media that consists of highly permeable medium made up either of rocks or plastic materials. As the wastewater flows over the surface of the media the organisms in the slime remove the organic matter from the flow (Metcalf and Eddy, 1972). The organisms aerobically decompose the solids producing more organisms and stable wastes, which either become part of the slime or are discharged back into the wastewater flowing over the media. The wastewater continues through the filter to the under drain system where it is collected and carried out of the filter. At the same time, air flows through the filter (bottom to the top or top to bottom depending on temperature) and oxygen is transferred from the air to the wastewater and slime to maintain the aerobic conditions. Periodically the slime on the media becomes too heavy and portions will be released. This material is carried out of the filter with the wastewater flow and is removed in the settling tank following the filter.

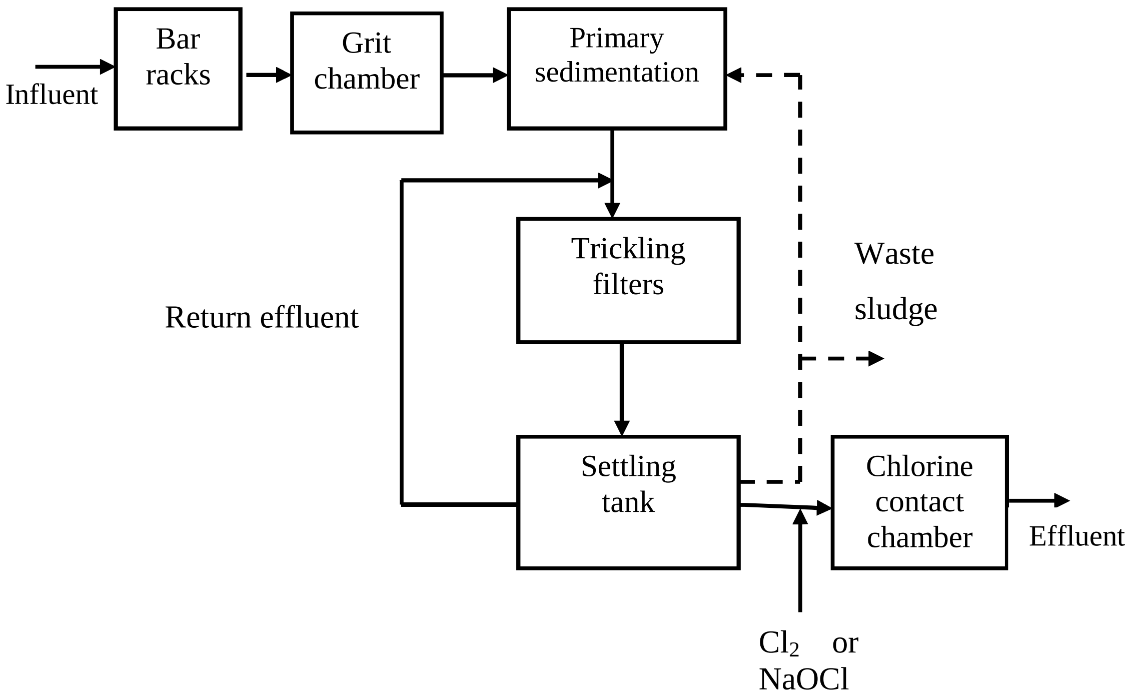


Figure 2-8 Trickling filter biological process for wastewater treatment (adapted from Metcalf and Eddy (1972)).

The water quality of treated effluent wastewater depends to a great extent on the quality of the municipal water supply, nature of the wastes added during use and the degree of treatment the wastewater has received (Pedrero et al., 2010). According to Lazarova and Bahri (2005), municipal wastewater that has limited industrial wastewater input generally contains concentrations of organic compounds that do not present any health concerns when used for irrigation. This implies that the source of the wastewater influences the quality of STSE and its use after treatment. Therefore, it is necessary to sample and analyse the treated effluent to ascertain its suitability for agricultural purposes.

2.6.3 Suitability of wastewater for irrigation

Adequate water supply of usable quality influences irrigated farming. Water quality concerns have often been neglected because good quality water supplies have been plentiful and readily available. Because of the global scarcity of good quality water and competing water demands in many areas this situation is now changing. As a result, the need for supplemental water sources in most cases from less desirable sources has become the likely available option. To avoid problems when using these poor quality water supplies, there must be sound planning to ensure that the quality of water available is put to the best use. **Table 2-1** summarises the guidelines to the quality of wastewater to be used in irrigated agriculture.

2.6.3.1 Chemical and physical characteristics of wastewater

Suitability of wastewater for irrigation depends on physical and chemical factors that are relevant in relation to yield and quality of crops, maintenance of productivity and protection of the environment. Wastewater quality or suitability is judged on the potential severity of the problems that can be expected to develop during long term usage (Ayers and Westcot, 1985). These problems are often caused by the characteristics of wastewater that includes suspended solids, salinity, pH, inorganic non-metallic constituents, nutrients, BOD and COD.

Total solids represent all of the solids in a wastewater sample remaining after wastewater has been evaporated off. Dissolved salts increase the osmotic potential of soil water and an increase in osmotic pressure of the soil solution increases the amount of energy which plants must expend to take up water from the soil. As a result, respiration is increased and the growth and yield of most plants decline progressively as osmotic pressure increases. Suspended solids can clog irrigation infrastructures especially if sprinklers or drip irrigation systems are used. In some cases if they are not biodegradable, the suspended solids can affect water percolation (WHO, 2006).

Alkalinity in wastewater results from the presence of the hydroxides, carbonates and bicarbonates of elements such as Ca, Mg, Na, K and NH_4^+-N. According to Metcalf and Eddy (1972), calcium and magnesium carbonates are the most common. Alkalinity in wastewater originates from water supply, groundwater and materials added during domestic water use. Knowledge of salinity of wastewater helps in determining leaching fraction and selection of the right cropping pattern.

Table 2-1 Guidelines for interpretations of water quality for irrigation. Adapted from Ayers and Westcot (1985)**.**

Potential Irrigation Problem				Units	Degree of Restriction on Use		
					None	Slight to Moderate	Severe
Salinity*(affects crop water availability)*							
	EC_w			dS/m	< 0.7	0.7 – 3.0	> 3.0
	(or)						
	TDS			mg/l	< 450	450 – 2000	> 2000
Infiltration *(affects infiltration rate of water into the soil. Evaluate using EC_w and SAR together)*							
SAR	= 0 – 3	**and EC_w**	=		> 0.7	0.7 – 0.2	< 0.2
	= 3 – 6		=		> 1.2	1.2 – 0.3	< 0.3
	= 6 – 12		=		> 1.9	1.9 – 0.5	< 0.5
	= 12 – 20		=		> 2.9	2.9 – 1.3	< 1.3
	= 20 – 40		=		> 5.0	5.0 – 2.9	< 2.9
Specific Ion Toxicity *(affects sensitive crops)*							
	Sodium (Na)						
	Surface irrigation			SAR	< 3	3 – 9	> 9
	Sprinkler irrigation			me/l	< 3	> 3	
	Chloride (Cl)						
	Surface irrigation			me/l	< 4	4 – 10	> 10
	Sprinkler irrigation			me/l	< 3	> 3	
	Boron (B)			mg/l	< 0.7	0.7 – 3.0	> 3.0
Miscellaneous Effects *(affects susceptible crops)*							
	Nitrogen (NO_3 - N)			mg/l	< 5	5 – 30	> 30
	Bicarbonate (HCO_3)						
	(overhead sprinkling only)			me/l	< 1.5	1.5 – 8.5	> 8.5
	pH				Normal Range 6.5 – 8.4		

The fertilising value of wastewater comes from the numerous nutrients contained. The suspended, colloidal and dissolved solids present in wastewater contain macro and micronutrients, which are essential for crop nutrition. N is the common nutrient in wastewater and has its source from either nitrogenous compounds of plant and animal origin, sodium nitrate or atmospheric N (Metcalf and Eddy, 1972). From secondary treated effluent, N ranges from 20 to 60 mg l^{-1} (FAO, 2003). P content in recycled water is too low to meet nutritional needs of plants (Lazarova and Bahri, 2005). However, P can accumulate and can affect future land uses as some plant species are sensitive to high P concentration.

2.6.3.2 Biological quality criteria

Sewage effluent contains pathogenic microorganisms like bacteria, viruses, fungi, algae, etc., having the potential risk to cause diseases and create immense harm to public health. The water borne diseases associated with wastewater are typhoid, paratyphoid fevers, dysentery and cholera, polio and infectious hepatitis. The Coliform group of bacteria comprises mainly species of the *genera Citrobacter, Enterobacter, Escherichia* and *Klebsiella* and includes Faecal Coliforms, of which *Escherichia coli* is the predominant species (WHO, 2006). They are not harmful but the presence of coliform groups of bacteria indicates the presence of pathogenic bacteria and fecal coliforms indicate fecal contamination and presence of enteric pathogens in surrounding water. Several coliforms are able to grow outside of the intestines, especially in hot climates. Hence their enumeration is unsuitable as a parameter. The fecal coliforms can grow at 44°C, so *E.coli*, is most satisfactory indicator parameter in sewage water use (Forslund et al., 2012).

Another common bacterium is the *Clostridium perfringens*. This bacterium is an exclusively faecal spore-forming anaerobe normally used to detect intermittent or previous pollution of water due to the prolonged survival of its spores (Pescod, 1992). According to Lazarova and Bahri (2005), potential risks induced by the presence of pathogenic microorganisms in wastewater or on crops may become actual risks if the following four criteria occur:

i. The pathogen must reach the plant or be able to multiply to the number required for an infective dose.
ii. A human host must come into contact with the effective dose of the pathogen
iii. The host must become infected
iv. Disease results from the infection or leads to further transmission.

Table 2-2 Survival times of various organisms in selected environmental media at 20 - 30ºC. (Source: WHO (2006)).

Organism	Survival times (days)		
	Fresh water and sewage	Crops	Soil
Viruses			
Enteroviruses	<120, usually <50	<60, usually <15	<100, usually <20
Bacteria			
Thermotolerant coliforms	<60, usually <30	<30, usually <15	<70, usually <20
Salmonella spp.	<60, usually <30	<30, usually <15	<70, usually <20
Shigella spp.	<30, usually <10	<10, usually <5	ND
V. cholerae	ND	<5, usually <2	<20, usually <10
Protozoa			
E.histolytica cysts	<30, usually <15	<10, usually <2	<20, usually <10
Cryptosporidium oocysts	<180, usually <70	<3, usually <2	<150, usually <75
Helminths			
Ascaris eggs	Years	<60, usually <30	Years
Tapeworm eggs	Many months	<60, usually <30	Many months

According to Sugiura (2009) wastewater can be a resource but it also can be a source of infectious agents and chemicals, thereby presenting a risk for public health. But according to Westcot (1997) the actual risk associated with irrigation with treated wastewater is much lower than previously estimated particularly with respect to bacterial pathogens. However, in order to protect farmers who are in contact with waste water (effluent), The World Health Organization (WHO, 2006), produced guidelines for wastewater reuse in agriculture. In the guidelines it is reported that increasing the quality of waste water by reducing the number of nematode eggs from 10 to ≤ 1 egg litre^{-1} resulted in very slight contamination of lettuce plants (0.3 eggs plant^{-1}).

Survival of these pathogens depends on a number of factors that include humidity, soil texture, temperature, plant type and pH (Jiménez, 2003). According to WHO (2006), the pathogens most resistant in the environment are helminth eggs that can survive in the soil for several years (

Table 2-2). Inactivation of pathogens is much more rapid in hot, sunny weather than in cool, cloudy or rainy conditions.

2.6.4 Irrigation methods

Selection of the appropriate irrigation system depends on the quality of wastewater, crop, tradition, background, skill, ability of the farmers to manage the different methods and the potential risk to the health of farmers, public and the environment. One major consideration is the salinity of the wastewater. The selected method should in particular include some precautionary measures towards minimising risks such as plant toxicity due to direct contact between foliage and the effluent, salt accumulation in the root zone, health hazards related to bioaerosol spraying, direct contact with the workers and infected product consumption (Pereira et al., 2002).

Wastewater irrigation in arid regions is largely influenced by salinity levels of the soil. Due to high rates of evaporation, huge quantities of salt accumulate in the soil. In this case incorporation of a leaching fraction when irrigation with STSE is essential to flush down the salts. However, including a leaching fraction has often led to over-irrigation, which may lead to excessive supply of nutrients to plants.

According to FAO (2003), drip irrigation is a safe and most promising method of irrigation with wastewater, particularly if purification is such to prevent extensive clogging. The frequent irrigations attainable by the drip irrigation results in more diluted salts in the soil moisture solutions and leaches salts out of the wetted root zone. However because of high investment costs most smallholder farmers cannot afford drip irrigation technology. **Table 2-3** summarises factors that affect choice of waste water irrigation technique and special measures when irrigating with sewage effluent.

The nature of sprinkler and micro-sprinklers irrigation makes these methods less appropriate to control health and contamination hazard as well as toxicity hazards (Pereira et al., 2002). In windy conditions there is a potential for the pathogens in wastewater to be carried away in the spray mist or in the formed aerosols with the wind drift and cause health hazard to the workers, farm population and the nearby residential areas (FAO, 2003).

Table 2-3 Selection of wastewater application techniques based on health protection (Source: WHO, (2006)**).**

Irrigation technique	Factors affecting choice	Special measures for wastewater
Flood	Lowest cost Exact levelling not required	Thorough protection for fieldworkers, crop handlers and consumers
Furrow	Low cost Levelling may be needed	Protection for fieldworkers, possibly for crop handlers and consumers
Spray and sprinkler	Medium water use efficiency Levelling not required Advanced sprinklers that reduce crop contamination and potential contamination of local communities have been developed that can reduce exposure to pathogens by 1 log unit	Some crops, especially tree fruits, are prone to more contamination Minimum distance of 50–100 m from houses and roads Anaerobic wastewaters should not be used because of odour nuisance New technologies reduce spray drift and may be able to reduce crop contamination by better targeting
Subsurface and localized (drip, trickle and bubbler)	High cost High water use efficiency Higher yields Potential for significant reduction of crop contamination Localized irrigation systems and subsurface irrigation can substantially reduce exposure to pathogens by 2–6 log units	Localized irrigation: selection of non-clogging emitters; filtration to prevent clogging of emitters

Due to the low investment costs associated with surface irrigation systems, most smallholder famers in developing countries have adopted water lifting technologies that best operate in conjunction with the surface irrigation methods. Water is lifted using treadle pumps, small motorised pumps and watering cans from rivers and water reservoirs into canals before being diverted to fields. With the usage of these water lifting technologies, smallholder farmers are in contact with water and that can increase the risk of health hazards if wastewater irrigation is employed. However, negative health effects are detected in association with usage of raw or poorly treated wastewater, while appropriate wastewater treatment provide for health protection (Pereira et al., 2002).

2.6.5 Crop water requirement under effluent irrigation

The consumptive use of water by plants is determined by crop and atmospheric factors. Climatic influence on loss of water though transpiration and evaporation are all considered when estimating crop water requirements. As the crop develops and leaves shades more and more of the ground, soil evaporation becomes more restricted and transpiration becomes the dominant process (Allen et al., 1998). Towards senescence, soil evaporation increases again as the plants shades off leaves.

Irrigation is tailor made to supply water equivalent to the crop water requirements of plants. There is a conflict on how much plant nutrients versus how much water the plant needs when wastewater is used. In supplying enough water to maintain desirable crop quality and yield, more nutrients than needed are applied, thus exceeding the agronomic rates or rate of nutrient use (Alshammary and Qian, 2008). This problem is compounded during the dry and hot seasons when the crop water requirements are high. Irrigation with STSE therefore presents a challenge as it can result in over or under supply of nutrients if irrigation is based on crop water requirement (nutrient imbalances). The extent of the over or under-supply of nutrients will depend on the nutrient levels in the wastewater, the nutrient demand of the crop (nutrient application rate) and the length of the growing period of the crop. When effluent (with low nutrient content) is irrigated to crops with a shorter growing season, the nutrient application rate is likely not met. While if the wastewater has a high nutrient content, then over supply of nutrients is a possibility. However, combining nutrient supply from compost and other nutrient suppliers (compost) can help in cases of a crop with a short growing season.

2.6.6 Nutrient dynamics associated with STSE irrigation

In effluent, a large proportion of effluent–N is composed of NH_4^+-N and NO_3^--N though a small percentage (about 10%) of effluent–N is organic–N (Fonseca et al., 2007b). The organic material in effluent consists predominately of dead algae with fast decomposition rates (Snow et al., 1999). NO_3^--N and NH_4^+–N concentrations in the soil may increase as a direct result of inorganic N in the effluent or indirectly through improved soil water status and increased soil organic matter (SOM) mineralisation (Livesley et al., 2007; Myers et al., 1982). Although nitrification in the soil can proceed within hours, NH_4^+-N can also be electrostatically adsorbed to the cation exchange complex that may delay microbial transformation processes (Fonseca et al., 2007a). Increasing mineralisation of soil organic N due to STSE application and lack of N synchronisation between crop N requirement and N input by effluent can result in NO_3^--N increase in the soil hence the potential for leaching. Though leaching is often associated with NO_3^--N, Livesley et al., (2007) in a field and lysimeter study on soil water NO_3^--N and NH_4^+-N dynamics under a sewage effluent–irrigated Eucalyptus plantation, concluded that application of effluent promoted downward movement of NH_4^+-N. This suggests that in certain conditions NH_4^+-N or organic N may be the dominant form of N to be leached. Silva et al., (2000) and Singleton et al., (2001) suggested that preferential flow is responsible for the downward movement of NH_4^+-N as it was initiated when soil water conditions reached field capacity.

The effectiveness of soil–plant systems to assimilate STSE applied N and P will depend on the biological, chemical and physical attributes of the soil, as well as plant uptake and irrigation management (Barton et al., 2005). STSE-applied N can be removed biologically via plant uptake (if the crop is harvested e.g. for making silage for animal feed), denitrification, volatilisation, or immobilisation into the soil organic matter. Any excess N remaining is likely to be leached. Furthermore, additional inorganic N may become available if irrigation increases net N mineralisation rates of soil organic matter (Polglase et al., 1995). P applied through STSE can be chemically adsorbed by the soil, taken up by P plants, or leached from the soil profile. The extent of all these processes will vary with soil texture. For example, denitrification is often greater in loamy-textured than sandy-textured soils (Parfitt and Salt, 2001), while according to Brennan et al., (1994) P retention increases with increasing iron oxides, aluminium oxides, and alumino silicate minerals.

In some instances, irrigation with STSE elevates exchangeable Na concentrations and exchangeable Na percentage in the soil. A 15 fold increase in exchangeable Na and 13-fold increase in exchangeable Na percentage were reported by Fonseca et al., (2005b). High Na concentrations in soils (sodicity) can cause deterioration of soil physical properties, specifically dispersion of clay with subsequent breakdown of soil structure, blocking of pores and decrease in soil permeability (Lado and Ben-Hur, 2009).

Blocking of soil pores affects the hydraulic conductivity of the soil (Lado and Ben-Hur, 2009).

Though other authors have noted increased pH after irrigation with treated effluent, the high soil buffering capacity plays a significant role to suppress the increase. As reported by Fonseca et al., (2005b), slight increase in pH can be attributed to (i) high effluent pH (ii) addition of exchangeable cations and alkalinity by effluent; (iii) increase of denitrification (Friedel et al., 2000) consuming one mol of H^+ for each mol denitrified NO_3^--N; (iv) addition of organic residues to the soil and (vi) interaction of these factors associated with low cation exchange capacity (CEC) of the soil (Fonseca et al., 2005b). However, after 20 years of effluent irrigation, Xu et al., (2010) observed a decrease in pH by 1.08 units while from plots irrigated for 3 and 8 years, soil pH was slightly affected. Similarly, Rattan et al., (2005) after two decades of STSE irrigation found a decrease in pH of about 0.4 units. The decrease in soil pH was consistent with the acidic pH values of the STSE. As reported by Xu et al., (2010), the long term change in soil pH is as a result of displacing cations, adding weak organic acids in soils or excessive leaching of basic cations.

2.7 Integrated nutrient supply

2.7.1 Compost and inorganic fertilisers

Integrated nutrient supply into the soil from compost and inorganic fertilisers though not well understood improves compost use efficiency by increasing mineralisation of compost-N, particularly in soils with low indigenous inorganic-N content (Han et al., 2004). Integrating readily available sources of inorganic-N with compost reduces the amount of compost applied. Reduction in quantity of compost applied also reduces labour costs associated with making the compost (in case of smallholder farmers), transportation and application of the compost. According to Sikora and Enkiri, (2001), if compost is applied to agricultural land at the N requirement of grain crops (40–100 kg N ha^{-1}), application rates approach 40–100 Mg ha^{-1} compost.

Nutrient integration from inorganic and organic sources can reduce the accumulation of non-nutrient constituents in soils mainly when bio-solids and sewage sludge compost are used as organic amendments. According to Palm et al., (1997), integrated use of organic and inorganic sources influences nutrient availability in the following ways;

i. by the nutrients added,
ii. through mineralisation – immobilisation patterns,
iii. as an energy source for microbial activities,
iv. as precursors to soil organic matter (SOM),
v. by reducing phosphorus (P) sorption of the soil.

The strength of the integrated nutrient input-system lies in its ability to meet the short-term as well as long-term nutrient requirements of crops through the fast-releasing fertiliser nutrient pool and the slow releasing organic nutrient pool, respectively (Sharma et al., 2008).

According to Han et al., (2004), there is a decrease in net N mineralisation during the first days of urea-compost (composted sawdust and manure) blending due to the growing microbial biomass as a result of the organic-C and inorganic-N in the compost. Despite that observation, Han et al., (2004) noted net mineralisation at the end of their 6 weeks incubation experiment ranging from 7.6% to 14.5% on three different soils incubated at 25 ± 0.5 °C. Similarly Goyal et al., (1999) reported increased microbial biomass C as a result of blending organic amendments with inorganic fertilisers from 147 mg kg^{-1} in unfertilised soil to 423 mg kg^{-1} in soils amended with wheat straw and inorganic fertilisers (urea and single super phosphate). The amendments provided readily decomposable organic matter in addition to increasing root biomass and root exudates due to greater crop growth. It is expected therefore that compost-fertiliser blends will have high efficiency in terms of supplying enough nutrients for crop growth.

Sikora and Enkiri (2000) in an 87-day growth chamber incubation study (fescue grass at 25°C) amended sandy loam soils with different combinations of biosolid compost and NH_4NO_3 fertiliser of 0/100%, 16.7/83.3%, 33/65% and 50/50% (supplying 100 mg kg^{-1} total N). They noted linear yield increment (9.1, 10.5 and11.4 g pot^{-1}) in blends with increasing percentages of biosolid compost N and decreasing percent fertiliser. Blends containing 33 or 50% compost produced yields greater than 100% NH_4NO_3. Sikora and Enkiri (2000) obtained comparable results for N uptake. They concluded that benefits from biosolids compost are seen when NH_4NO_3 contribution in the blends is 67% or less than the recommended for optimal growth. The limit set by Sikora and Enkiri (2000) on the nutrient blending can be disputed as availability and uptake of nutrients for crop growth depends on a number of factors including the initial conditions of the soil, length of the growth season and the physiology of the crop.

In a 6 year field study on loamy sand soils, Eichler-Lobermann et al., (2007) concluded that organic (cattle manure and manure biowaste compost) and inorganic fertilisation (triple-superphosphate) resulted in high contents of water soluble P and double lactase soluble P in the soil. Mixing organic and inorganic fertilisation in spring resulted in high spring wheat yield of up to 7.7 t ha^{-1} as compared to autumn wheat yield of 7.3 t ha^{-1}. Similarly, nutrient uptake of up to 150 kg ha^{-1} total P was observed for spring wheat as compared to 143 kg ha^{-1} in autumn (Eichler-Lobermann et al., 2007). They suggested that availability of P depends on a number of factors that include pH, organic matter content and soil moisture. Eichler-Lobermann et al., (2007) did not explore the effect that these factors may have had on the availability of P in the soil and plant uptake in the experiment.

2.7.2 Compost and effluent nutrient integration

The concept of nutrient blending from organic and inorganic sources can advance the idea of sustainable agriculture with less input of inorganic fertilisers and higher build-up of organic matter in the soil through organic amendments. Much as the synergistic effects have been observed of compost on inorganic fertilisers and vice versa (Sharma et al., 2008), inorganic (chemical) fertilisers still remain expensive to most smallholder, urban and peri-urban farmers in developing countries because of the escalating energy costs associated with its production, cost of transportation and above all, according to Herring and Fantel, (1993) and Hilton et al., (2010) P-fertilisers may run out of supply as presently known reserves of rock phosphates may be depleted within 50 years.

Sikora and Enkiri (2000), integrated bio-solid compost and NH_4NO_3 in a growth cabinet experiment, Eichler-Lobermann et al., (2007) integrated organic (cattle manure and manure biowaste compost) and inorganic fertilisation, Goyal et al., (1999) combined wheat straw, urea and single super phosphate while Han et al., (2004), integrated urea and compost (composted sawdust and manure). A seemingly viable option is to blend compost with STSE for irrigation of crops but no research has been done on this type of nutrient integration. STSE is a product of sewage treatment that is either disposed in rivers or into the soil. Disposal of effluent in most developing countries is regarded as an economically viable option as it reduces the cost of treating sewage (Friedel et al., 2000). According to Katanda et al., (2007), sewage effluent contains larger proportions of plant nutrients such as N, P, organic matter as well as heavy metals. Heavy metals are non-biodegradable and can persist in the environment long enough to diminish soil quality and to be taken up by plants into the food chain (Katanda et al., 2007; Chakrabarti and Chakrabarti, 1988). However, once treated, STSE contains a lower proportion of heavy metals as most of the heavy metal end up in sludge hence the use of STSE for irrigation of crops (Emongor and Ramolemana, 2004).

The issues raised above can be attributed to among other factors, the effluent loading (application) rates, the sewage treatment process and the concentration of the heavy metals in the effluent. Usage of sewage effluent as the sole source of nutrients for crops results into larger loading rates that will lead into accumulation or leaching of heavy metals and plant nutrients in the soil.

In optimising nutrient potential from compost through irrigation with treated effluent, various questions will arise, one of which will be how to combine organic amendments with readily available nutrient sources e.g. effluent and organic amendments to optimise nutrient availability from the later. The proportion of organic amendments (compost) to the quantity of STSE is a research question that has to be answered so as not to deprive the crops of nutrients and water. Synchronisation of available and nutrient uptake is another significant issue that often affects integration of nutrient sources.

The contribution of effluent-N to the N cycle has been presented in **Figure 2-9.** It also shows the processes that N undergoes for plant and microbial availability. Effluent-N will contribute to the N cycle through either the NH_4^+-N or NO_3^--N pools; in the NO_3^--N pool it will be immediately available for plant uptake or leached while in the NH_4^+-N pool, immobilisation, nitrification or plant uptake by higher plants will be expected to take place.

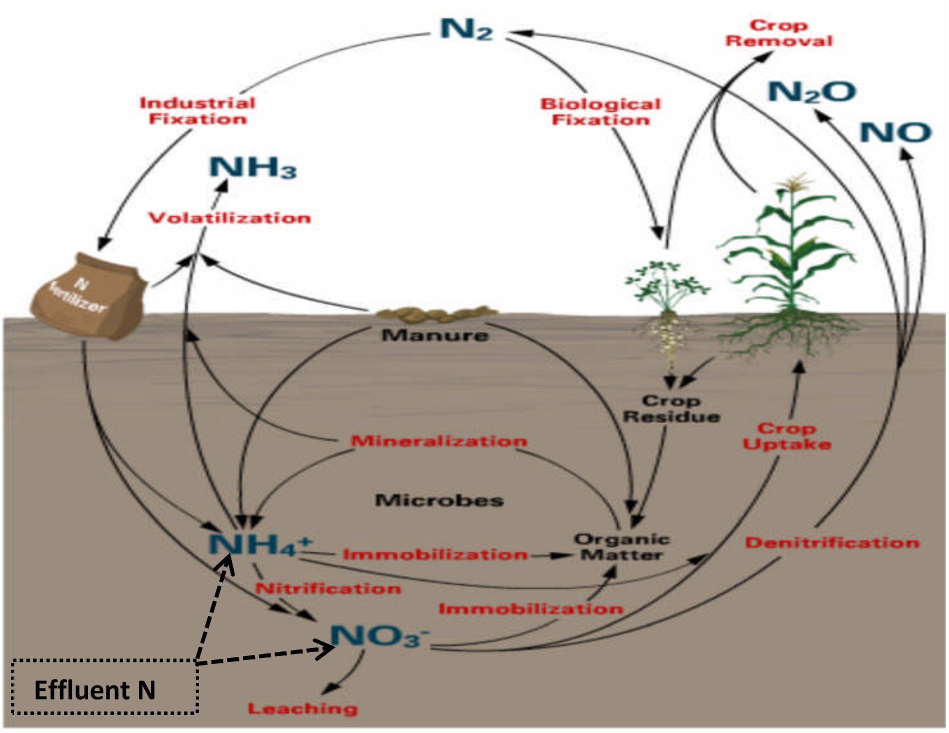


Figure 2-9 Modified N cycle showing the contribution of effluent to the N-cycle (adapted from Johnson et al., (2005)).

Optimising nutrient potential without proper estimation of the rates of mineralisation of the compost due to the readily available nutrients from treated effluent can result in the accumulation of nutrients into the soil and leaching in case of excess precipitation or irrigation. Determination of N mineralisation rates is essential in estimating how much of the organic amendments to apply in order to supply the right amount of nutrients for optimising crop production. In optimising nutrient potential from compost by irrigating compost amended soils with treated effluent, the study of nutrient dynamics due to the conjunctive supply of nutrients becomes necessary for efficient sustainable management of the system. Excess NO_3^--N which can come from the optimised nutrients of compost and treated effluent can be lost by denitrification and can lead to undesirable N-outputs from the soil e.g. in the form of gases (NO_x and N_2O) which are known to increase the greenhouse effect (Fonseca et al., 2007b). Residual effects, percolation and subsequent accumulation should also be considered in terms of heavy metals. Though it is expected that the treated effluent is likely to contain less heavy metals, subsequent impacts of the these metals can trigger a chain reaction in the soil that can affect availability of other essential nutrients.

Compost blending with effluent (treated) can be regarded as a lifeline to most urban dwellers in developing countries who practise urban or peri-urban agriculture. According to FAO (1998) "urban" agriculture implies growing crops, raising small livestock or milk cows within the city for own-consumption or sale while "peri-urban" agriculture refers to farm units close to towns which operate intensive, semi - or fully commercial farms to grow vegetables and other horticultural crops, raise chickens and other livestock and produce milk and eggs.

2.8 Mechanisms of nutrient uptake by plants

2.8.1 Ryegrass nutrition

Ryegrass is probably the most widely used grass for turf purposes and it is thought to be one of the first cultivated grasses (Beard, 1972). Performance of ryegrass depends on its fibrous and extensive root system with a large surface area which is ideal for nutrient uptake.

Ryegrass, just as other turf grasses require N in largest amounts as they contain between 3 to 6% N on a dry matter basis provided there is no soil N deficiency (Beard, 1972). Ryegrass is considered a heavy user of N. As a result, after each cut, N deficiencies are likely to occur as N loss is high due to plant uptake and the demand for N is high for regeneration. This explains why Labuschagne (2005) applied fertiliser five days after each ryegrass cut. To avoid nutrient deficiencies after cutting ryegrass, there is a need to therefore apply fertilisers or organic amendments. According to Xu and Phillips (1999), N nutrition affects ryegrass in a number of ways including (a) shoot growth (b) root growth (c) shoot density (d) colour (e) disease proneness (f) heat, cold and drought hardiness and (g) recuperative potential. Though ryegrass responds vigorously to N fertilisation, uncontrolled level of N nutrition results into restricted depth and extent of the root system thereby decreasing nutrient and water uptake (Beard, 1972).

Perennial ryegrass emergence is inhibited by elevated levels of NH_4^+-N or total N. It is also ecologically significant that high NH_4^+-N can inhibit seed germination and seedling establishment (Britto and Kronzucker, 2006). According to Barker (2001) seedling emergence in soils amended with compost (NH_4^+-N levels exceeding 600 mg N ha^{-1}) was only 74% of that in soils amended with compost with lower NH_4^+ concentration (68 mg N ha^{-1}). Cao et al., (2011) concluded that optimal total, shoot and root dry matter production can be achieved if ryegrass is supplied with close to equal amounts of NO_3^--N and NH_4^+-N. However, availability of ample supply of water even without N addition can produce greater dry matter. When water supply is plentiful, yield increase at all N levels to a greater extent than when water is in short supply (Ehlers and Goss, 2003). It is likely that large quantities of available N are formed by stimulated mineralisation of soil-borne N, the large pool of N bound in organic matter.

The quantity of P used by ryegrass is less than the amount of N and K. In ryegrass, P is vital particularly during seeding stage to ensure rapid establishment and stimulated root growth. According to Beard (1972), turf grasses respond to phosphorous applications when the soil P level is less than 0.005 g kg^{-1}, while K is required in relatively large amounts though less than N requirement. K accumulates in young, actively growing grasses and decreases rapidly with age. An increase in K content decreases the degree of tissue hydration in times of water stress. K is directly involved in maintaining water status of ryegrass, turgor pressure of cells and the opening and closing of stomata.

2.8.2 Movement of nutrients to root surfaces

Nutrient mobility in the soil is important in relation to nutrient availability to plants. Nutrients in the soil solution are absorbed by roots of growing plants. As plants grow, roots proliferate through the soil and move into areas that previously were occupied by soil containing available nutrients. In this way, root surfaces intercept nutrients and absorb nutrients during root development.

According to Marschner (1995), amount of nutrients intercepted by the roots depends on (a) the amounts of available nutrients in the soil volume occupied by roots; (b) root volume as a percentage of the total soil volume and (c) the proportion of total soil volume occupied by pores (dependent on soil bulky density). Because roots grow through soil pores which may have higher than average nutrient content, it is estimated that roots would contact a maximum of 3% of the available nutrients in the soil (Tiquia et al., 2002; Tisdale et al., 1990).

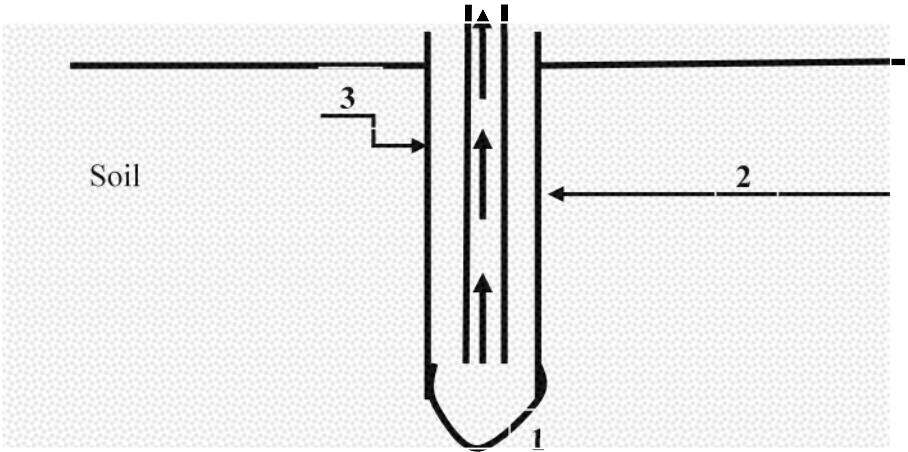


Figure 2-10 Movement of mineral elements to the root surface of soil-grown plants. 1-Root interception: soil volume displaced by root volume. 2-Mass flow: transport of bulk soil solution along water potential gradient. 3- Diffusion: nutrient transport along concentration gradient. (Source: Marschner (1995)).

Mass flow of water and dissolved nutrients (**Figure 2-10**) in the soil is driven by transpiration. As plants transpire, a gradient is created that allows water to flow towards the roots with nutrients ions where the nutrients are absorbed by the roots. Strebel and Duynisveld (1989) estimated that mass flow can account for up to 33% of the total N transported to the roots in sugar beet and cereal crops during the period of maximum demand by the crop. The proportion of mass flow contribution to nutrient transport in maize is in the order Ca > Mg > N >S > K > P ≈ Mn ≈ Zn ≈ Cu ≈ Fe with the mean values for the first five as 100, 63, 56, 45 and 10% respectively (Oliveira et al., 2010).

The quantity of nutrients supplied to plants by mass flow is based on the nutrient concentration in the soil solution and the amount of water transpired either per shoot tissue or per hectare of a crop (Marschner, 1995). According to Tisdale et al., (1990) nutrients that are not held tightly by the soil (e.g. Ca^{+2}, Cl^{-1}, Mg^{+2} and NO_3^{-}) and those that are in abundance are supplied by mass flow. Nutrient flux towards roots by mass flow and diffusion enhance uptake of mineralised N by plants mainly greater moisture values (Ehlers and Goss, 2003).

Diffusion transport allows nutrients to move from areas of high to low concentration. Around the roots of an actively growing plant, nutrients are depleted thereby creating a zone of low concentration as compared to the surrounding areas. The created diffusion gradient moves nutrients from the surrounding areas to the roots.

Table 2-4 Annual average concentration of mineral nutrients in the soil solution (top soil, 0-20 cm) of arable soil (Luvisol, pH 7.7)[a].

Concentration (µm)								
K	Ca	Mg	NH_4-N	NO_3-N	SO_4-S	PO_4-P	Zn	Mn
510	1650	490	48	3100	590	1.5	0.48	0.002

[a] Adapted from Marschner (1995).

The concentration of nutrients in the soil solution is of primary importance for nutrient supply to the roots. However soil nutrient concentration is affected by a number of factors; soil moisture, soil depth, pH, cation-exchange capacity, redox potential, quantity of soil organic matter and microbial activity, season of the year and fertiliser or organic amendment application (Tisdale et al., 1990).

The concentration of nutrients in the soil solution is an indicator of mobility of nutrients towards the root surface or in a vertical direction (leaching). Phosphorous concentration in the soil is extremely low (**Table 2-4**) such that leaching and or transport by mass flow is generally of minor importance in conventional farming. Phosphate strongly interacts with surface-active sequioxides and oxidhydrates of clay minerals (Marschner, 1995). However, in nutrient interaction systems involving compost and STSE, phosphorous dynamics may change due to addition of effluent-P. This change in dynamics can result in accumulation in the soil or leaching of P to groundwater bodies.

Moisture content therefore plays a bigger role in nutrient transfer to plant roots and affect dry matter production. As illustrated by Eck (1988), with no water shortage, one unit of N fertiliser generates a higher yield increase than at limited water supply. According to Ehlers and Goss (2003) reduced soil moisture will not only decrease the nutrient flux from the soil reserves towards the roots (nutrient availability) but also the sink of nutrients (nutrient uptake by plants).

2.8.3 Plant uptake of nitrogen

As discussed earlier on, soil moisture helps in nutrient transfer to plant roots. Nutrient diffusion gradient is created around plant roots once nutrients are absorbed across the root surface. Because of its primary role as an obligate constituent of proteins, nucleic acids, and many secondary products, plants typically contain up to 4% of their dry weight in the form of N (Glass, 2009). In the case of N, the usual sources of N to plants are NO_3^--N and NH_4^+-N. N uptake has been used previously by Chadwick et al., (2000) as an indicator of N dynamics (N mineralisation and immobilisation).

N availability is the main factor determining crop production. As stated by Lewis (1986), factors that influence uptake of NO_3^--N and NH_4^+-N are:

a) Availability of energy-rich compounds
b) Soil temperature
c) Soil pH.

Nitrate uptake by root cells from the cell walls through the plasma membrane to the symplasm as described by Kirkby et al., (2009) is an "uphill" process requiring energy. N uptake is regulated by crop demand which is determined by shoot-root interactions in which nitrate acts as a signal for metabolism and root development (Kirkby et al., 2009; Antille, 2011). This means that root tips, especially lateral roots, convert the nitrate signal into root growth responses (Forde, 2002).

N can be taken up by crops either as ammonium or nitrate (Tisdale et al., 1990). Though much focus has been on nitrate uptake, Glass (2009) indicated that even for plants growing in regions where nitrate predominates still typically take up ammonium at higher rates than nitrate, when both forms are present. In some forests where available N concentrations greatly exceed those of NH_4^+-N, NO_3^--N uptake is blocked by the inhibitory effect of the presence of ammonium (Gessler et al., 1998). NH_4^+-N uptake has been reported to be highly competitive in depressing uptake of the main mineral cations, K^+, Ca^{2+} and Mg^{2+} (Kirkby, 1968). But these ions are largely without effect on the uptake of NH_4^+-N (Marschner, 1995). Tisdale et al., (1990) alluded to the fact that ammonium can be replaced by cations that expand the lattices of clay minerals (K^+, Ca^{2+}, Na^+ and Mg^{2+}).

2.8.4 Nitrogen use efficiency

The response to N application by plants is assessed using various indices and methods. Though most of the indices fail to take into account all the factors affecting the efficient utilisation of N; their usage provides enough information to help improve nutrient–use efficiency. As summarised by Johnston and Poulton (2009), basing on the work of Cassman et al., (1998), the commonly used methods and indices are;

a) Direct method: The direct method is ideally used in cases where the fertiliser N is labelled with the heavy isotope ^{15}N. In this case, it is possible to measure N in the crop, in the harvested product and also the N remaining in the soil. However, according to Antille (2011) it is generally acknowledged that the direct method provides accurate estimates of N use efficiency though the main disadvantage of the ^{15}N experiments are the associated costs.

b) Difference Method: The major requirement of this method is that the experiment should have treatments with and without added N and the data generated is used to calculate;

- NUE using yield: Considered as the “agronomic efficiency” of applied N, it is calculated by considering yields in treatments with and without N application (**Equation 2-4**);

$$A_e = \frac{(Y_N - Y_0)}{F_N}$$ **Equation 2-4**

  Where Y_N and Y_0 are the crop yields with and without applied N respectively while F_N is the amount of N applied, all in Kg ha^{-1}.

- NUE using N uptake: Considered as the “apparent recovery” or “apparent efficiency” of applied N, it is calculated by considering N uptake in treatments with and without N application (**Equation 2-5**);

$$R_N = \frac{(U_N - U_0)}{F_N}$$ **Equation 2-5**

Where U_N and U_0 are the N uptake by the crop corresponding to treatments with and without applied N respectively while F_N is the amount of N applied, all in Kg ha^{-1}.

Apart from the above indices, two other commonly used indices are (Johnston and Poulton, 2009; Cassman et al., 1998; Zhu et al., 2011);

- Partial factor productivity (PFP_e) of applied N; It is defined as the kg product produced per kg increase in N in the crop (**Equation 2-6**). It is calculated as,

$$PFP_e = \frac{Y_N}{F_N} \qquad \textbf{Equation 2-6}$$

Where Y_N and F_N are as described above.

- Physiological efficiency (P_e) of applied N; It is defined as the kg product increase in N in the crop (**Equation 2-7**). It is calculated as,

$$P_e = \frac{(Y_N - Y_0)}{(U_N - U_0)} \qquad \textbf{Equation 2-7}$$

Where Y_N, Y_0, U_N and U_0 are as described above.

2.9 Conclusion

This literature review has provided a broad overview of the issues related to production and disposal of compost and STSE, soil fertility decline and critical assessment of ways to replenish soil fertility. It has also provided concepts on composting, composting methods and wastewater irrigation. Above all, the overview given above has exposed the challenges associated with organic amendment application to soil. Similarly, the advantages and disadvantages of sewage effluent irrigation have also been presented and discussed.

This literature review has also exposed gaps in knowledge that, even though compost and mineral fertiliser integration has been studied, it appears integration of compost and STSE has not been studied before. Much as various researchers have worked on nutrient integration focusing on compost and inorganic fertilisers, adoption of this type of nutrient integration by smallholder farmers can be affected by the inability of smallholder farmers to purchase inorganic fertilisers. An understanding of nutrient dynamics, dry matter production, nutrient accumulation in the soil and environmental protection will be essential to fill the gaps in knowledge that currently exists in context of compost and sewage effluent nutrient integration. Better understanding of the fate of nutrients, especially N and P from compost amendment and STSE irrigation is critical to properly manage integrated compost and STSE nutrient application. Understanding nutrient dynamics in compost amended soils and soil irrigated with STSE is essential in order to meet crop nutrient requirements whilst ensuring minimum environmental impacts from nitrate and phosphorous leaching. Consequently, the aim of this research is to optimise nutrient potential from compost and irrigation with wastewater (STSE) in order to meet the nutritional requirements of crops for sustainable crop production and environmental protection. The experimental work to achieve the aim of the research has been presented in **Chapter 1** (**Section 1.3**).

3 INCUBATION STUDY

This chapter presents and discusses the incubation experiments that were carried out in 2010. A static non-leached soil incubation system was used without plants. The incubation experiments were conducted to determine the impact of the combination of compost and treated effluent on N and P dynamics and microbial biomass as reported in **Chapter 1**. More emphasis was placed on N dynamics and N mineralisation in the incubated soils.

3.1 Introduction

Soil incubation has been widely used for assessing N availability characteristics of organic amendments with the potential for use in crop production (Bitzer and Sims, 1988). According to Smith et al., (1998), there are various methods through which the accumulation of NO_3^--N, NH_4^+-N and N dynamics can be studied. Leaching procedures and static soil incubation are some of the methods. Static soil incubation is the most preferred. In leaching procedures, N mineralisation is under estimated due to losses of soluble potentially mineralisable N in leachate (Garau et al., 1986). However, Garau et al., (1986) noted that the presence of large concentrations of inorganic N in non-leached systems can disturb the mineralisation process and under-predict the potentially mineralisable N content of an organic amendment. In particular, large concentrations of NH_4^+-N may be inhibitory to nitrification processes as excess NH_4^+-N can be toxic to *Nitrobacter* (Brady and Weil, 2008). The maximum concentration of NH_4^+-N reported in field soil tolerated by nitrifying organisms is between 400 mg kg^{-1} (McIntosh and Frederick, 1958) and 800 mg kg^{-1} (Broadbent et al., 1957) as reported by Smith et al., (1998) in a field experiment.

Understanding the mechanisms of interaction of compost and STSE on N dynamics as a result of nutrient integration of compost and STSE required a controlled experimental procedure in which N could be monitored whilst keeping constant soil moisture content and temperature. The outcomes of this study will provide an understanding of nutrient availability as a result of nutrient integration of compost and sewage effluent. The general trends of the results of incubation study will therefore provide insights to the pot and lysimeter studies. The specific objectives of the incubation study are presented below;

i. To examine the effects of combined application of compost and STSE on N availability with time (dynamics) in arable soils.
ii. To study the influence of STSE on the mineralisation of organic matter in arable soil.
iii. To determine the impact of the combination of compost and treated effluent on microbial biomass C (MBC) and microbial biomass N (MBN).

3.2 Materials and Methods

3.2.1 Description of soils

The soils used for the incubation experiment were a sandy loam and clay loam. The soils were bought from a commercial soil company (Boughton Loam and Turf Management Limited in Kettering, UK). The same soils were also used for the pot (glasshouse) study in **Chapter 4**. The soils were selected as they had distinctive chemical and physical characteristics. Soil texture was verified by analysing the soil samples using the pipette method (Avery and Bascomb, 1982; BSI, 1990). Before the textural analysis, the soils were air dried and ground to pass through a 2 mm sieve.

Field capacity was measured on a soil sample of known volume that was fully wetted and then allowed to sit under free drainage for two to three days (Hillel, 1980; Carter and Gregorich, 2008). After free drainage had ceased, the water content of the sample was determined. The estimation of field capacity helped to decide on the maximum possible quantity of STSE that could be combined with the compost.

3.2.2 Description of compost

Greenwaste compost used in the incubation experiments was sourced from MEC Recycling Ltd in Lincolnshire, UK and it was publicly available specification (PAS 100) accredited and was of very good quality (Simmons, 2013). The same batch of compost was also used for the pot (glasshouse) study (**Chapter 4**). Greenwaste compost at MEC Recycling Ltd is processed using the windrow composting system. Windrow composting involves piling of waste in long narrow rows or piles. A detailed description of compositing methods has been presented in **Chapter 2**.

The compost was air dried and sieved to pass a 2 mm sieve before amendment. Compost samples were analysed to establish background characteristics that have been reported in **Section 3.3.1.**

3.2.3 Description of STSE

The source of the STSE was the Cranfield University Sewage Treatment Plant (CUSTP). CUSTP has a design capacity of 2000 population equivalent (Sugiura, 2009) and processes municipal sewage from the university campus. At the plant, a trickling filter system is used to treat sewage. The CUSTP has the following major components; settlement tank, dosing chamber, biodek filter, humus tank and tertiary filter (**Figure 3-1**). In the tertiary filters, much of the residual suspended matter is removed. Treated effluent from the treatment plant is discharged into Chicheley brook.

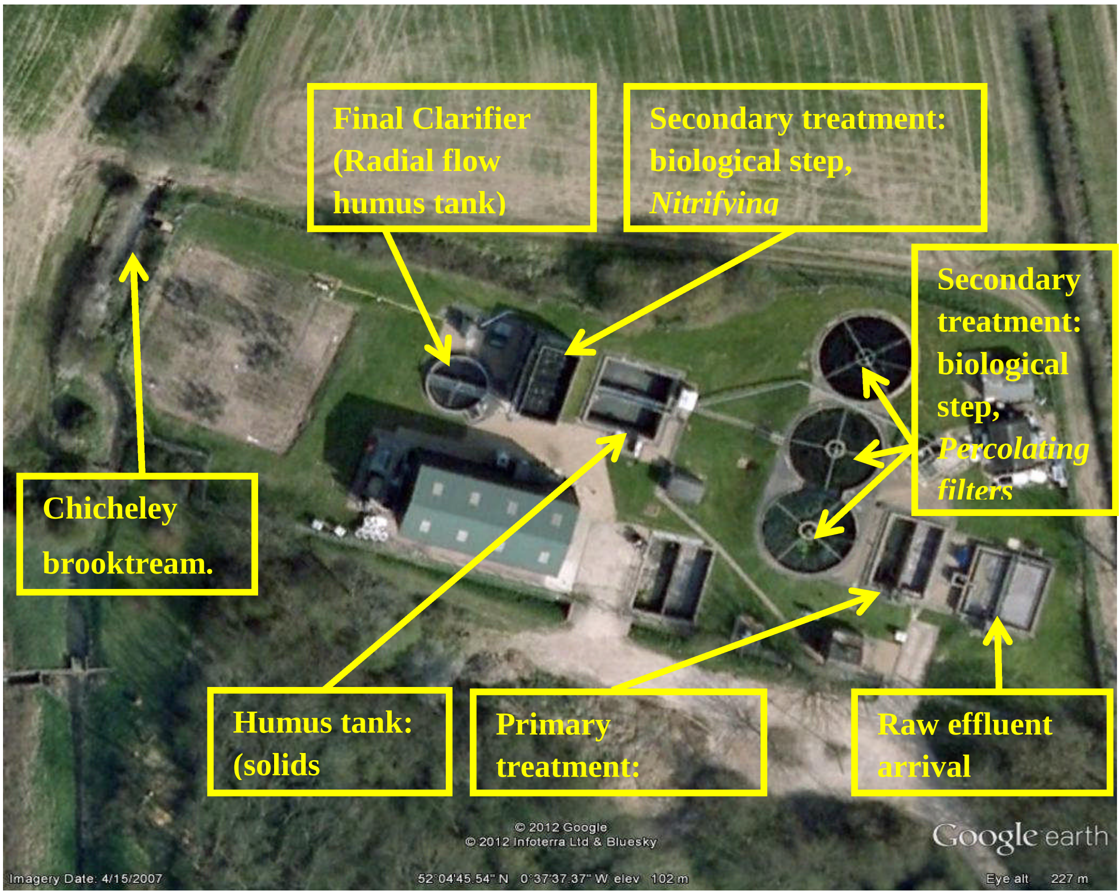


Figure 3-1 Cranfield sewage treatment plant showing the various sewage treatment processes and stages (Adapted from Sugiura (2009)**, courtesy of Google earth).**

Nutrient characterisation of the STSE was done by using reactive kits (spectroquant Merck® test kits). Spectroquant tests depend on biochemical reactions yielding a coloured product enabling determination of various nutrients in sewage effluent. The STSE was filtered before adding to respective cell tests and mixing with reagents for determination dissolved NH_4^+ - N, NO_3^-- N, K and PO_4^{3-}-P. For total dissolved N and P, STSE was digested by heating at 120°C for 60 and 30 minutes respectively before adding to total N and total P tests kits. A Merck spectroquant Nova 60 photometer was used to read the concentration of the nutrients using the bar code on the cell tests kits. The results of chemical and physical analyses of STSE have been presented in **Section 3.3.1**.

3.2.4 Soil incubation

The incubation involved the use of triplicate samples in a randomised block design of sandy loam and clay loam soils. 300 g of soil was packed into 0.5 l incubation pots with a bulk density of 1300 kg m^{-3}. The soils were mixed thoroughly with greenwaste compost and wetted-up with 119 ml deionised water (for the control and treatments with compost alone) and STSE (**Table 3-2**). The incubation pots were covered with aluminium foil perforated to allow gaseous exchange. The samples were incubated in a growth chamber in the dark at 25°C for a period of 120 days. To minimise the evaporation losses of water from the incubation pots and also to regulate humidity, a water bath was placed at the bottom of the incubator. The pots and the incubation chamber are shown in **Figure 3-2**.

To supply equal amounts of N through STSE for corresponding treatments in clay loam and sandy loam soil, an average of the two field capacities for the clay loam and sandy loam soil was used. This resulted in moisture content of 100 and 98% of maximum water holding capacity (WHC) for treatments in sandy loam and clay loam soil respectively. The moisture content of the incubated soil samples was extended to maximum water holding capacity (field capacity) so as to accommodate the nutrient (N) application rates of 37.5, 75 and 150 kg total N ha^{-1} whilst avoiding water saturation of the soils. Other authors have used moisture contents of below field capacity in the incubation studies. Smith et al., (1998) incubated soil samples at 40% WHC while Serna and Pomares (1992) adjusted soil moisture content to a lower level of 75% of field capacity. Moisture content of the incubated soil samples was maintained by regular weighing and replacing the evaporated soil water with deionised water (Fonseca et al., 2007b; Haer and Benbi, 2003).

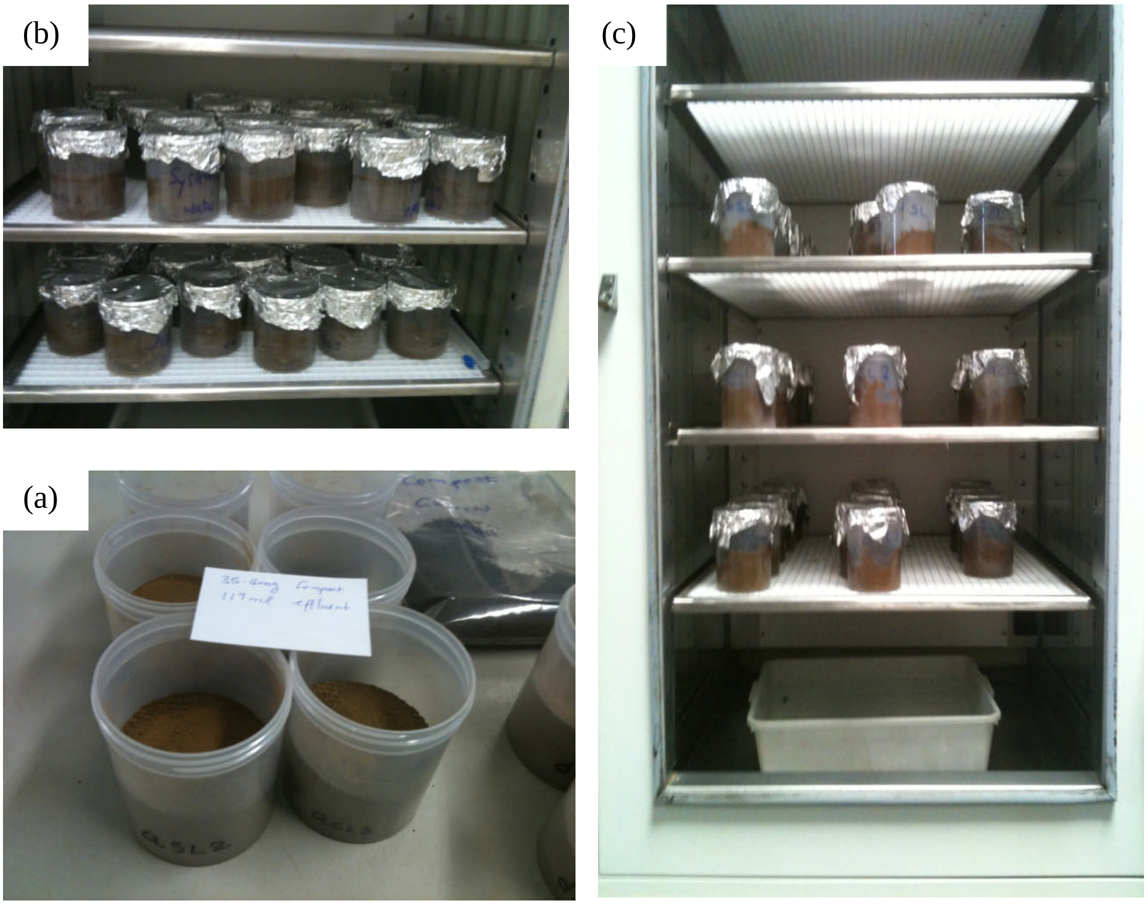


Figure 3-2 Incubation study showing a) preparation of incubation pots, b) and c) incubation pots and plastic tray to maintain humidity inside the incubator.

Nutrient supply combinations from compost and STSE were developed as treatments to supply 37.5, 75 and 150 kg N ha^{-1} on the sandy loam and the clay loam soils by compost, STSE or a combination of the two. The highest rate of N application in the incubation experiments (150 kg N ha^{-1}) was selected to approximate ryegrass N requirement for a single cut (MAFF, 2000). In the other experiments (pot and lysimeter), ryegrass was grown as a test crop. Using identical N application rates for all experiments enabled linkage and information supply between the experiments.

Table 3-1 Nutrient supply combinations and application rates

Treatment combinations (kg N ha^{-1})	N application rate (kg N ha^{-1})		Compost (ton ha^{-1})	Effluent (ml kg^{-1})
	Compost	Effluent		
$0_{compost} + 37.5_{effluent}$	0	37.5	0	396
$37.5_{compost} + 37.5_{effluent}$	37.5	37.5	2.3	396
$112.5_{compost} + 37.5_{effluent}$	112.5	37.5	6.8	396
$75_{compost} + 0_{effluent}$	75	0	4.6	0
$150_{compost} + 0_{effluent}$	150	0	9.1	0
Control	0	0	0	0

Five treatments, compost-effluent nutrient integration (($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ +$37.5_{effluent}$)) supplying 75 kg N ha^{-1} and 150 kg N ha^{-1} respectively, STSE (($0_{compost}$ + $37.5_{effluent}$)) supplying 37.5 kg N ha^{-1} and compost alone (($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ +$0_{effluent}$)) supplying 75 and 150 kg N ha^{-1} respectively. A control (non-amended treatment) was used as a reference treatment**. Table 3-1** and **Table 3-2** outline the treatments and the quantity of STSE and greenwaste compost applied to supply 37.5, 75 and 150 kg N ha^{-1}. Calculation of the quantity of compost to apply per pot was based on an assumption of compost application depth of 15 cm and a soil bulk density of 1300 kg m^{-3}.

Table 3-2 Quantity of compost and STSE applied in each treatment combination of compost and STSE.

Treatment (kg [N] ha^{-1})	Compost (mg beaker^{-1})	STSE (ml beaker^{-1})
$0_{compost} + 37.5_{effluent}$	0	119
$37.5_{compost} + 37.5_{effluent}$	354	119
$112.5_{compost} + 37.5_{effluent}$	1046	119
$75_{compost} + 0_{effluent}$	700	0
$150_{compost} + 0_{effluent}$	1400	0
Control	0	0

3.2.5 Measurement and analysis

Soil samples were taken from the incubated soils at the start and once every 30 days for a period of 120 days. Soil sampling was non-destructive as such the remaining soil after a sampling event was used for subsequent sampling events until the end of the incubation experiment. Soil samples were taken from the incubation pots without thoroughly mixing all the soil in the pots. Although mixing of soils during sampling allows for a thorough and homogeneous mixing of the soil, it can promote N mineralisation due to additional air (oxygen) introduced during in the process. At each sampling time, NH_4^+-N, NO_3^- -N, pH, MBN and MBC were analysed from the soil samples while soil mineral N was estimated as the sum of NO_3^--N and NH_4^+-N. Total N, total C, extractable P and total P were analysed at the start and end of the incubation.

NO_3^--N and NH_4^+-N was determined using the *Burkard Scientific Segmented Flow Analyser*. Sample extraction was done on 20 g fresh soil; 100 ml of 2 mol L^{-1} potassium chloride (KCl) solution was used to extract the sample before filtration using Whatman No.4 filter paper (MAFF, 1986a). Soil pH was determined in 1:5 soil/water extracts (BSI, 2000c) while in STSE, pH and electrical conductivity (EC) were measured by *Jenway 4400* pH and conductivity meter. Measurements of TN and TC in soil and compost were made at the start and end of the study on fine ground dried samples by catalytic tube combustion using the *Vario EL III CHNOS* elemental analyser (BSI, 2000b). Soil samples were acid–digested using the aqua regia process for the determination of TP_{soil} (BS EN 13657, 2002). Determination of plant extractable P in soil was conducted using sodium hydrogen carbonate (BSI, 1995). Soil organic matter was determined on dehydrated air dried soil by loss-on-ignition (BSI, 2000a). Cation exchange capacity of the soil was determined using barium chloride (BSI 7755., 1996).

Microbial biomass extraction was done using the chloroform fumigation-extraction procedure (BSI 7755:, 1997). Extractable C and N were determined using the *Segmented Flow Analyser*. At each sampling time, MBC and MBN were calculated as the difference between extractable C and N in fumigated and non-fumigated samples respectively. The differences for biomass C and N were divided by k-factors of 0.38 and 0.45 respectively (Logah et al., 2011; Liu et al., 2007). The k-factors are used to account for the fraction of the killed biomass extracted as C or N.

Net N mineralisation as a result of the compost amendment and sewage effluent was calculated based on the accumulation of mineral N in the soil. Net mineralised N was calculated as the difference in the concentration of mineral N between the treatments and the control (Han et al., 2004). Net N mineralisation has been presented as a percentage of the total N applied (**Equation 3-1**).

Equation 3-1

$$NM_{net} = \frac{\text{Mineral N}_{\text{amended soil}} \text{-Mineral N}_{\text{control soil}}}{\text{Total N}_{\text{applied}}}$$

Where;

NM_{net} = Net N mineralisation from a unit of applied N (kg inorganic N kg^{-1} applied N)

Mineral $N_{\text{amended soil}}$ = Total mineral N in amended soil

Mineral $N_{\text{control soil}}$ = Total mineral N in control soil

Total N_{applied} = Applied total N

To model N dynamics, the first order equation with one mineralisation pool proposed by Stanford and Smith (1972) as cited in Cordovil et al., (2005) was fitted to the mineral N data to estimate the mineralisable N from each treatment (**Equation 3-2**).

$$N_m = N_0 \left(1 - \exp\left[-kt\right]\right)$$ **Equation 3-2**

In the first order model, N_m represent the accumulated mineralised N at time *t*, while *k* is the mineralisation rate constant and the amount of potentially mineralisable N is given by N_0. This model was chosen so as to estimate the potentially mineralisable N as a result of the various nutrient amendment combinations.

3.2.6 Statistical analysis

The effect of each treatment and the influence of soil type, application rates and compost-STSE combinations on the measured variables with time were assessed by repeated measures analysis of ANOVA (General Linear Models) in Statistica 9.0 to determine significant difference of means. Significantly different levels of treatments were identified using least significant differences at a probability of 0.05 (Fishers LSD).

Normal probability plots were used to assess whether or not a data set was normally distributed. Occasionally extreme values were removed during the statistical analyses. Model fitting was done using non-linear estimation in Statistica 10 and a t-test was used to compare the values of N_o for each treatments.

3.3 Results and Discussion

3.3.1 Soil, compost and STSE characteristics

3.3.1.1 Soil

Initial analyses of clay loam and sandy loam soil prior to the start of the experiment showed that the clay loam soil was more fertile than the sandy loam soil. The soil pH for clay loam and sandy loam was 7.6 and 6.8 respectively. Clay loam soil had an initial higher NO_3^--N of 65 mg kg^{-1} as compared to sandy loam soil of 5.8 mg kg^{-1}. Organic C in clay loam was 2.9% while in sandy loam it was 1.6%. Cation exchange capacity (CEC) in sandy loam and clay loam was 9.7 and 17 cmol+ kg^{-1} respectively. A summary of results of the analyses conducted on the soils prior to the incubation experiment has been presented in **Table 3-3**. The C/N ratios of the sandy loam and the clay loam soils were 10.8 and 11.9. These soils were typical UK soils with lower C/N ratios.

3.3.1.2 Compost

The results of the initial analysis of chemical and physical properties of greenwaste compost have been presented in **Table 3-4**. Greenwaste compost was rich in mineral N (NH_4^+-N + NO_3^--N) with 57% of mineral N in NH_4^+-N form. NH_4^+-N content in compost is used to ascertain the maturity of compost. NH_4^+-N presence is an indication of unstabilised materials mostly as a result of using raw materials with low C/N ratio. As reported by Zucconni and De Bertoldi (1986), immature compost has NH_4^+-N exceeding 0.04% dry weight. The level of NH_4^+-N in the compost was 0.048% dry weight which was on the limit of compost maturity. However total N in the greenwaste compost was 1.65% which was above the minimum required for mature compost of 0.6% dry weight (Zucconi and De Bertoldi, 1986).

Table 3-3 Soil characteristics prior to the beginning of the incubation experiments. Values in parenthesis are standard error of the means (SEM) with n = 4.

	Sandy loam	SEM	Clay loam	SEM
Extractable P (mg kg^{-1})	39.7	0.7	21.5	0.77
Organic matter (%)	3.7	0.10	4.8	0.08
TP (g kg^{-1})	0.84	0.03	0.61	0.01
pH	6.8	0.05	7.6	0.03
NH_4^+-N (mg kg^{-1})	2.67	0.23	3.7	0
NO_3^--N (mg kg^{-1})	5.81	0.19	65.1	1.14
Total C (%)	1.29	0.02	1.68	0.02
Organic C (%)	1.6	0.04	2.88	0.07
TN_{soil} (%)	0.12	0	0.14	0
CEC (cmol+ kg^{-1})	9.7	0.18	17	0.15
C/N ratio	10.8	0.08	11.9	0.15
Sand (%)	77	1.8	42	0.4
Silt (%)	11	2.1	29	1.1
Clay (%)	12	0.2	29	0.7

Table 3-4 Greenwaste compost properties prior to the start of the incubation experiment (n = 4).

	Compost	SEM
Extractable P (mg kg^{-1})	367	56
Organic matter (%)	38	0.98
Dry Matter (%)	58	1.73
TP (g kg^{-1})	2.8	0.19
pH	7.9	0.01
NH_4^+ -N (mg kg^{-1})	476	97
NO_3^- - N (mg kg^{-1})	359	76
TC_{comp} (%)	21.5	0.54
Total N (%)	1.65	0.05
C/N ratio	13	0.13

The C/N ratio of greenwaste compost was 13, indicating a possibility of N mineralisation and N availability for plant growth under optimum soil and environmental conditions. Application of compost with C/N ratio greater than 15 results in limited availability of N in the soil due to N immobilisation (Gutser et al., 2005).

3.3.1.3 Secondary treated sewage effluent

The results of the analyses of STSE are presented in **Table 3-5**. Total N in the STSE was predominantly in the form of mineral N (NH_4^+-N and NO_3^--N) with dissolved organic N contributing about 17% of total N. The pH of the treated effluent was neutral with an electrical conductivity of 764 µS cm^{-1}. Compared to **Table 2.1** (Literature review), electrical conductivity of the STSE was within the 0.7 - 3.0 dS m^{-1} category, indicating that the STSE had slight to moderate restriction for irrigation. NO_3^--N concentration in the STSE was within the category 5 – 30 mg l^{-1}, indicating again that the STSE had slight to moderate restriction for irrigation.

Table 3-5 Chemical and physical properties of the STSE for the incubation experiment (n = 3).

	PO_4^{3-}	K	TN	NH_4^+ - N	NO_3^--N	P	Conductivity	pH
	------------------------------mg l^{-1}------------------------------						(µS cm^{-1})	
Effluent	14.2	4.9	45.7	9.7	27.7	32.9	764	7
± SEM	0.1	0	4.3	0.1	0.4	1.0	0.3	0

3.3.2 Nitrogen release characteristics

The results in this section have been presented as NO_3^--N and NH_4^+-N accumulation/release with time in the soils. Mineral N (NO_3^--N and NH_4^+-N) has also been presented in this section. The results of potential N mineralisation of the sandy loam and the clay loam soils have been presented and also discussed in this section.

3.3.2.1 Nitrogen dynamics

Statistical analysis indicated that NO_3^--N accumulation during the incubation study was significantly higher in treatments with combinations of compost and STSE in the clay loam as compared to the same combinations in sandy loam soil ($p < 0.05$). In the clay loam, mean NO_3^--N release aggregated across the combinations of compost and STSE, N application rates and time was 94 mg kg^{-1} while in the sandy loam it was 17 mg kg^{-1}. Significant differences were found between the combination of soil type and compost-STSE combinations ($p < 0.05$) and interaction of soil type, compost-effluent combinations and application rate ($p < 0.05$). As shown in **Figure 3-3** and **Figure 3-5,** the three way interaction of soil type, compost-effluent combinations and application rate and incubation time was significantly different ($p = 0.00$).

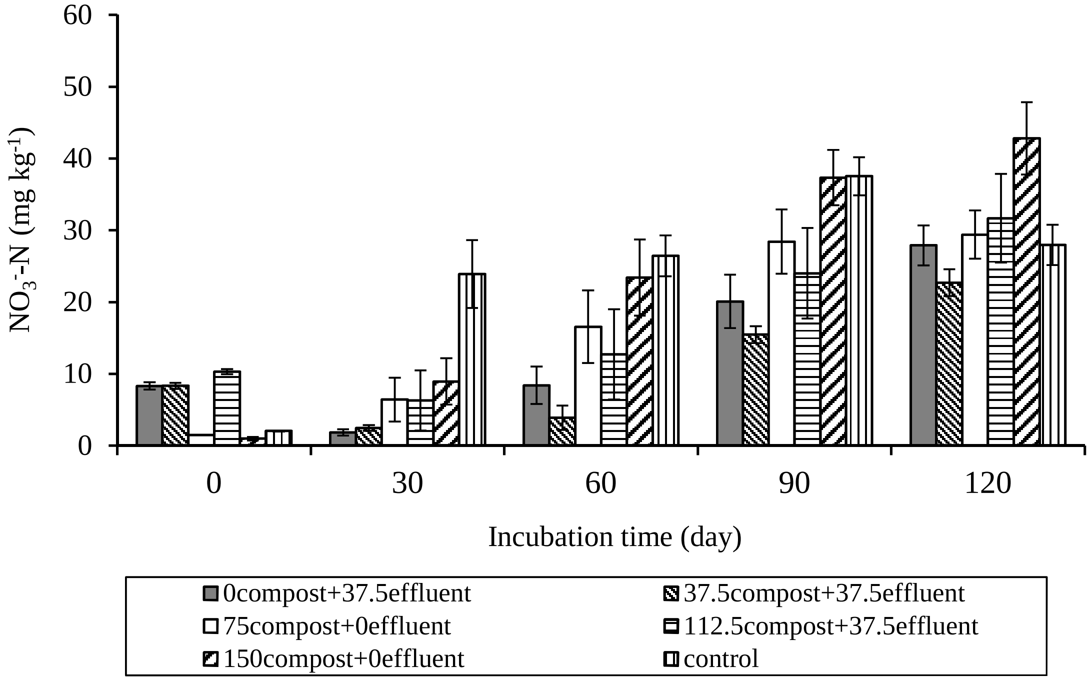


Figure 3-3 Release of NO_3^--N during the incubation experiment as influenced by soil type, N application rates and combinations of compost and STSE in the sandy loam ($p = 0.00$). Error bars represent for ± standard error of the means (SEM).

In the sandy loam (**Figure 3-3)**, the treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) showed rapid decline of NO_3^--N in the first 30 days. For the treatments ($0_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$), NO_3^--N declined from 8.3 to 1.8 mg kg^{-1} and 10.3 to 6.3 mg kg^{-1} respectively while for the treatment ($37.5_{compost}$ + $37.5_{effluent}$), NO_3^--N declined from 8.3 to 2.5 mg kg^{-1}. However, in the control soil between 30 to 90 days, NO_3^--N was significantly higher ($p < 0.05$) than in treatments with nutrient integration and effluent alone. The low C/N ratio of the sandy loam soil (10.8) guaranteed N mineralisation from the control as C/N ratio of 15 is a critical limit separating soil groups with higher or lower N release (Springob and Kirchmann, 2003).

In terms of release rates of NO_3^--N (**Figure 3-4**), treatments with STSE alone and combination of compost and STSE showed N immobilisation (denoted by negative NO_3^--N values) in the first 30 days. After 30 days, NO_3^--N started to accumulate at rates of 0.2, 0.05 and 0.15 mg kg^{-1} day^{-1} for the treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) respectively. Unlike to treatments in the clay loam, the NO_3^--N only started decline around 120 days.

Since denitrification losses were likely avoided as the moisture content of the incubated soil was maintained at field capacity, N immobilisation was the likely cause of the decline in NO_3^--N. Other studies have shown that there is either considerable depression of N mineralisation or a net loss of mineral N in soils wetter than optimum (Myers et al., 1982). Guntiñas et al., (2012) found that in laboratory conditions optimal moisture content for N mineralisation was 80% of the field capacity and mineralisation at 100% of field capacity was only slightly lower than that obtained at 80%.

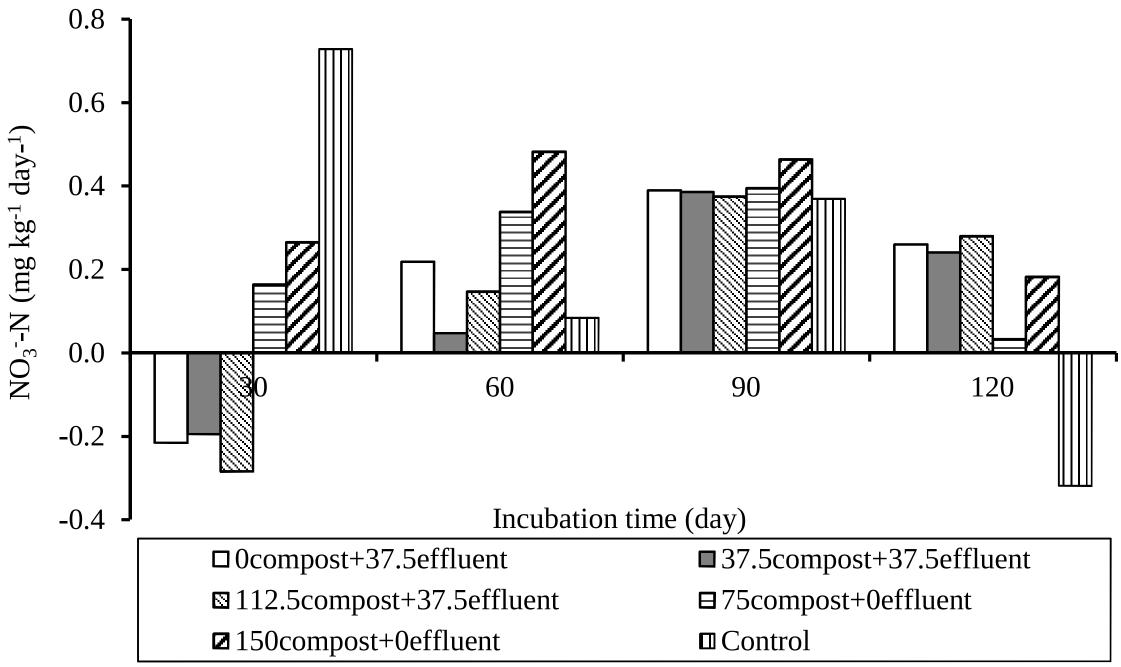


Figure 3-4 NO_3^--N release rates as influenced by incubation time and combinations of compost and treated STSE in the sandy loam soil.

Rowel (1994) suggested that losses of N by denitrification are minimised when the soil is maintained at approximately 60% of field capacity. But in non-leached incubation systems, water moves down through gravity and settles at the bottom of the incubation pots. A moisture profile is therefore created in the incubated soil with the soil at the bottom wetter than at the top. The moisture profile is usually related to accumulation of soil mineral N in soil. Antille (2011) concluded that this moisture profile often results in localised anaerobic conditions in the soil which promotes N losses through denitrification. The reduced rates of NO_3^--N release towards the end of the incubation study in **Figure 3-4** was possibly due to N_2O loss through denitrification.

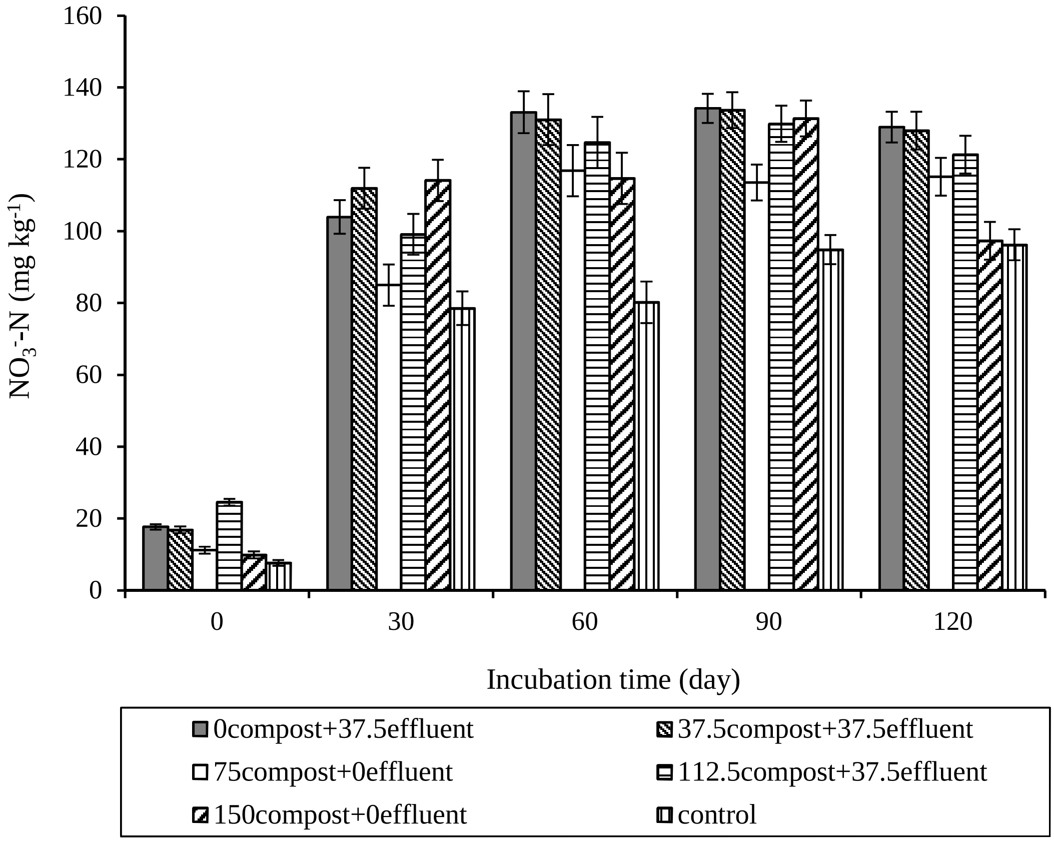


Figure 3-5 Release of NO_3^--N during the incubation experiment as influenced by soil type, N application rates and combinations of compost and STSE in the clay loam (p = 0.00). Error bars represent for ± SEM.

In clay loam soil, NO_3^--N significantly increased in the first 30 days (**Figure 3-5**). NO_3^--N increased significantly from 25 to 99 mg kg^{-1} in the first 30 days for the treatment ($112.5_{compost}$ + $37.5_{effluent}$), after which the increase was at a reduced rate to 125 and 130 mg kg^{-1} soil on day 60 and 90 respectively. On day 120, NO_3^--N reduced to 121 mg kg^{-1}. A similar trend was observed for all treatments with a contribution of STSE N (($112.5_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($0_{compost}$ + $37.5_{effluent}$)) but the changes in NO_3^--N were significant with time ($p < 0.05$).

The rate of N mineralisation reduces when the easily decomposable fraction of the organic matter is used up by microbes. For the treatment ($0_{compost}$ + $37.5_{effluent}$), during the first 30 days NO_3^--N accumulation was at a rate of 2.9 mg kg^{-1} day^{-1} before reducing to 1.0 mg kg^{-1} day^{-1} between 30 to 60 days. Similarly for the treatment ($37.5_{compost}$ + $37.5_{effluent}$) in the first 30 days, the rate of accumulation of NO_3^--N was 3.2 and 0.6 mg kg^{-1} day^{-1} between 30 to 60 days (**Figure 3-6**).

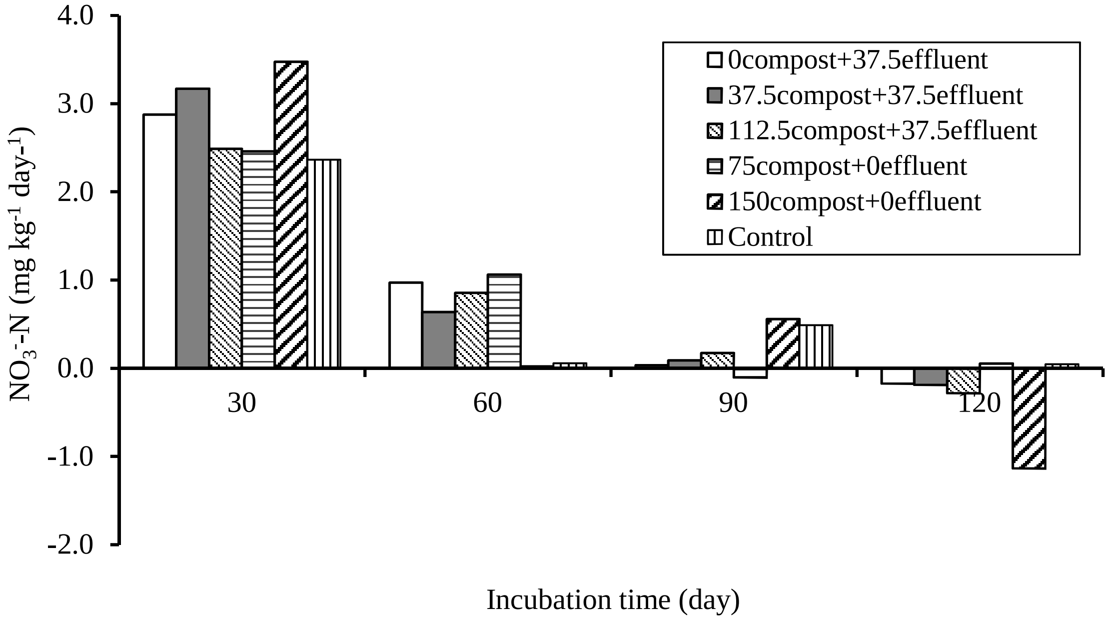


Figure 3-6 NO_3^--N release rates as influenced by incubation time and combinations of compost and STSE in the clay loam soil.

With respect to the two soils, during the first 30 days, the mean rate of NO_3^--N release in clay loam soil was 2.8 mg NO_3^--N kg^{-1} N day^{-1} while for sandy loam it was 0.1 mg NO_3^--N kg^{-1} N day^{-1}. However, between day 30 and 60 the rate reduced to 0.6 mg NO_3^--N day^{-1} for clay loam while for the sandy loam, it increased to 0.23 mg NO_3^--N kg^{-1} N day^{-1}. Comparing the two soils with respect to treatments with STSE or compost and STSE combinations, NO_3^--N release rate was largely higher in the first 30 days in clay loam. In sandy loam, NO_3^--N accumulated up until 90 days (**Figure 3-4**) though the release rates were significantly lower for all combinations of compost and STSE. The higher rates of NO_3^--N release in clay loam were largely because of the higher NO_3^--N concentration in the initial conditions of the soil (**Table 3-3**. In clay loam, the rapid mineralisation in the initial days utilised readily available and dissolved organic N by microbial biomass after which recalcitrant organic compounds were left in the soil.

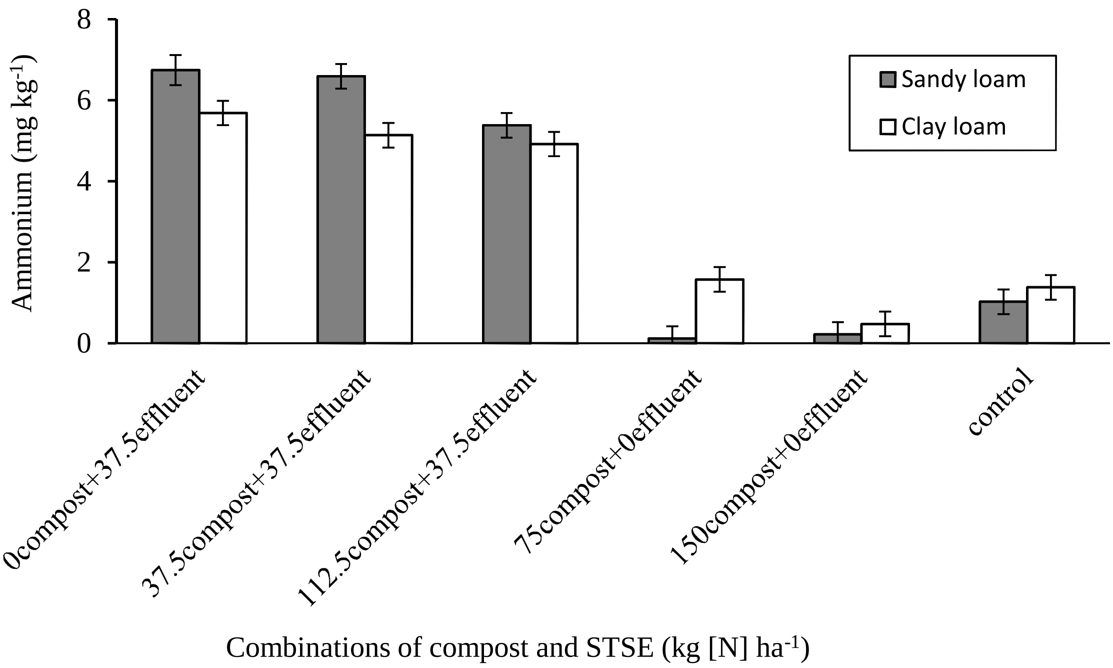


Figure 3-7 Mean Ammonium content (for the N application rates) during the first 30 days of the incubation experiment as influenced by the combination of compost and STSE in the sandy loam and clay loam soils (p = 0.001). Error bars represent for ± SEM.

The contribution of NH_4^+-N was only significant up to day 30 of the incubation after which its concentration was untraceable in all treatments. Statistical analysis of NH_4^+-N was therefore conducted only for the first 30 days (for soil samples collected on day 0 and 30). At the start of the experiment for the treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) in clay loam, NH_4^+-N concentration was 23%, 35% and 30% of inorganic N respectively before it became undetectable. This was an indication that the process of nitrification was rapid during the early stages of the incubation. Analysis of NH_4^+-N showed significant differences between the interactions of all treatment factors (**Figure 3-7**). In both soil types, NH_4^+-

N was significantly higher ($p < 0.05$) in treatments with either STSE alone or STSE in combination with compost. STSE influenced the concentration of NH_4^+ -N in the soil. NH_4^+-N was significantly lower in treatments with compost alone, ($75_{compost} + 0_{effluent}$) and ($150_{compost} + 0_{effluent}$) in the sandy loam and the clay loam soils (**Figure 3-7**). The low CEC reported in **Section 3.3.1.1** for sandy loam implied that NH_4^+-N could not be held up hence higher NH_4^+-N concentration in sandy loam soil. Duong et al., (2012) reported increased CEC in soils with 22% clay compared to soils with less clay content (13%). Sandy loam and clay loam soils used in the incubation experiment had 12% and 29% clay content respectively (**Table 3-3**), creating a possibility of increased CEC in clay loam compared to sandy loam soil.

The decline of NO_3^--N after 30 days in sandy loam (for treatments with STSE and STSE in combination with compost) coincided with the decline in NH_4^+-N. Since these were also the treatments with higher NH_4^+-N content, its reduction affected the accumulation of NO_3^--N. Through the process of nitrification, NH_4^+-N is nitrified to NO_3^--N however NH_4^+-N is also taken up by microbes (Han et al., 2004). NH_4^+-N has been described as the most preferred inorganic N by microbes (Fonseca et al., 2007b; Haer and Benbi, 2003). In case of N immobilisation, availability of NH_4^+-N for nitrification is limited hence reduced accumulation of NO_3^--N in the soil.

Soil mineral N in the compost-STSE nutrient combination treatments during the incubation is presented in **Table 3-6 and Table 3-7**. Soil mineral N was significantly influenced by the soil type and the combination of the effect of compost-STSE combination and the application rate. In sandy loam soil, the mean mineral N during the incubation was largely not significantly different. Compost and STSE integration did not influence the mean mineral N averaged across the sampling times however increasing the contribution of compost increased the mean mineral N in the treatments ($p > 0.05$). In both soil types, the trends of mineral N were similar to those of NO_3^--N largely because of the disappearance of NH_4^+-N after 30 days of the incubation.

In sandy loam, mineral N from the control soil was found to increase considerably with incubation time (**Table 3-6**). In some instances it was significantly higher than the treatments. A possibility of N release from soils has been related to soil's C/N. It was reported by Springob and Kirchmann (2003) that a C/N ratio of 15 is a critical limit separating soil groups with higher or lower N release. The sandy loam soil used in the incubation study had C/N ratio of 10.8 ± 0.08 (**Table 3-3**).

Table 3-6 Mean mineral N concentration in compost-STSE combinations in the sandy loam soil.

Compost-STSE Combinations	Mineral N (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}+37.5_{effluent}$	20.6^{a}	2.1^{a}	10.0^{abc}	22.6^{a}	29.0^{ab}	16.9^{ab}
$37.5_{compost}+37.5_{effluent}$	20.8^{a}	3.2^{a}	4.1^{c}	15.5^{a}	22.7^{ab}	13.2^{b}
$75_{compost}+0_{effluent}$	1.5^{bc}	6.8^{a}	16.8^{ab}	28.6^{ac}	29.4^{a}	16.6^{ab}
$112.5_{compost}+37.5_{effluent}$	20.3^{a}	7.0^{a}	12.7^{bd}	24.0^{a}	31.7^{a}	19.1^{ab}
$150_{compost}+0_{effluent}$	1.2^{bc}	8.9^{a}	23.4^{e}	37.3^{b}	42.8^{bc}	22.7^{ab}
Control	4.1^{bc}	23.9^{b}	26.4^{e}	37.5^{b}	28.0^{a}	24.0^{a}

Mineral N values followed by different letters in a column are significantly different ($p < 0.05$).

Table 3-7 Mean mineral N concentration in compost-STSE combinations in the clay loam soil.

Time (days)	Mineral N (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}+37.5_{effluent}$	28.8^{ac}	104.2^{aef}	133.1^{de}	134.2^{a}	^{cdeb}128.9	105.8^{a}
$37.5_{compost}+37.5_{effluent}$	29.0^{adef}	108.9^{aef}	130.7^{bef}	137.1^{a}	^{cde}135.4	108.2^{ac}
$75_{compost}+0_{effluent}$	14.2^{bcd}	85.7^{bd}	116.8^{bf}	113.5^{c}	^{b}115.2	89.1^{b}
$112.5_{compost}+37.5_{effluent}$	34.9^{ae}	99.5^{decf}	124.7^{bdef}	129.9^{a}	^{cde}121.3	102.1^{ac}
$150_{compost}+0_{effluent}$	10.7^{bcf}	114.1^{af}	114.7^{bcf}	131.4^{a}	^{ae}97.3	93.6^{b}
Control	10.1^{bc}	78.5^{be}	80.2^{a}	94.9^{b}	^{b}96.2	72.0^{b}

Mineral N values followed by different letters in a column are significantly different ($p < 0.05$)

In the clay loam soil, higher mean mineral N was observed in treatments with nutrient contribution from STSE. Significantly higher mean mineral N of 106, 108 and 102 mg kg^{-1} (**Table 3.7**) was observed for the treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) respectively. However, it should be noted that these treatments had different application rates of 37.5, 75 and 150 kg N ha^{-1} respectively. No significant differences were observed between the treatments with compost alone (($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ + $0_{effluent}$)) and the control.

3.3.2.2 Nitrogen mineralisation

Net N mineralisation (NM_{net}) was significantly higher ($p < 0.05$) in the clay loam as compared to the sandy loam soil. It was affected by the addition of STSE as well as the textural characteristics of the soils used. Significant increase in NM_{net} in the clay loam soil was observed for the ($0_{compost}$ + $37.5_{effluent}$) treatment (**Figure 3-8**). NM_{net} increased from 0.9 kg inorganic N kg^{-1} applied N (kg inorganic N per kg of applied N) at the start to 2.5 kg inorganic N kg^{-1} applied N after 60 days. Net N mineralisation reduced from 2.5 to 1.9 and 1.6 kg inorganic N kg^{-1} applied N on day 60, 90 and 120 respectively. The initial mineralisation for this treatment was significantly higher ($p < 0.05$) than the rest of the treatments.

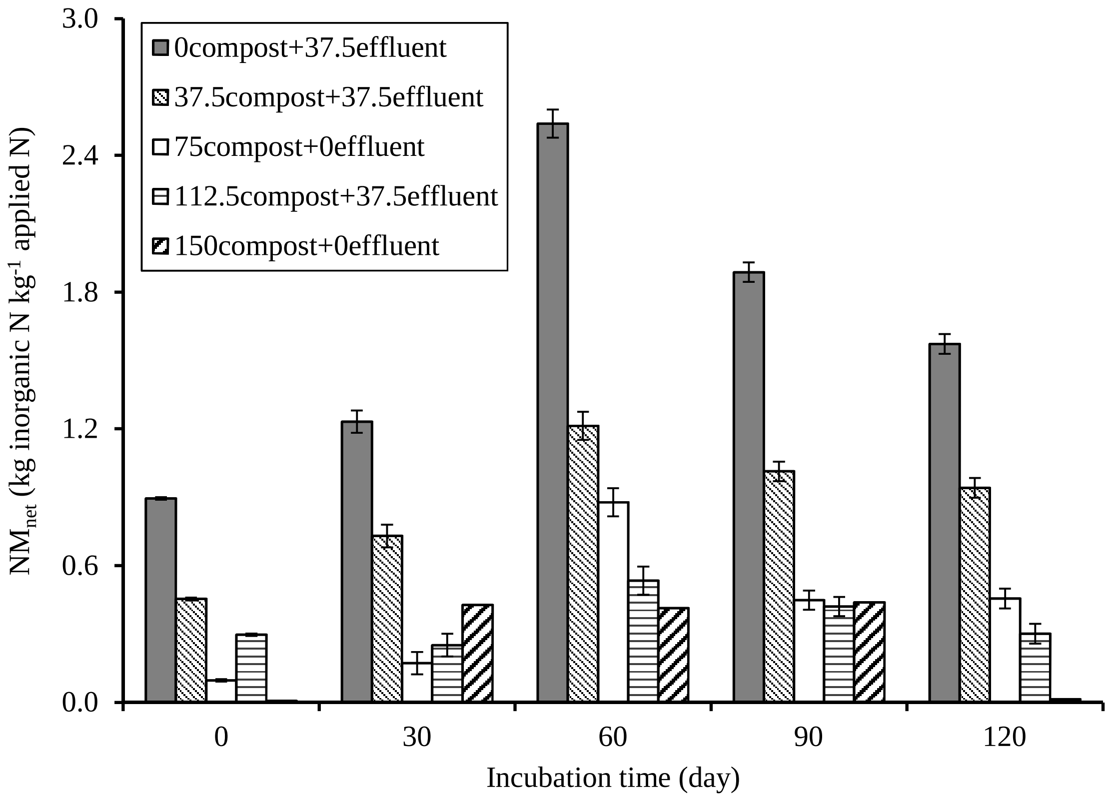


Figure 3-8 Effect of compost, STSE and combinations of compost and STSE amendments on net N mineralisation (NM_{net}) in the clay loam soil ($p = 0.00$). Error bars represent for ± SEM.

Higher NM_{net} was observed in treatments with either STSE or combination of compost and STSE apart from the treatment ($112.5_{compost}$ + $37.5_{effluent}$). In the clay loam, increasing the contribution of compost in a treatment resulted in a decrease in NM_{net}. Duong et al., (2012) reported increased CEC in soil with higher clay content. The highly decomposed organic matter in compost has a large number of cation binding sites (Duong et al., 2012) that increases the likelihood of NH_4^+-N adsorption. On day 60, NM_{net} was significantly higher for the treatment ($0_{compost}$ + $37.5_{effluent}$); for the ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) treatments it was 1.21 and 0.53 kg inorganic N kg^{-1} applied N respectively. On day 90, it was 1.01 and 0.42 kg inorganic N kg^{-1} applied N for the ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) treatments respectively (**Figure 3-8**).

It is worth noting that NM_{net} for the treatment ($0_{compost}$ + $37.5_{effluent}$) in clay loam from day 30 to the end of the incubation was significantly higher (**Figure 3-8**) than all the other treatments. The observed NM_{net} released from the ($0_{compost}$ + $37.5_{effluent}$) treatment was higher than the applied total N. On day 120, NM_{net} was 1.57 kg inorganic N kg^{-1} applied N. When similar observation were made by Azeez and Van Averbeke (2010), Eneji et al., (2002), Abbasi and Khizar (2012), Mubarak et al., (2010) and Khalil et al., (2007), they attributed the extra N mineralisation to the release of initially immobilised N due to death of microbes and also from microbial cell metabolism. Azeez and Van Averbeke (2010) observed up to 400% of total N mineralised when they amended sandy clay loam soil with goat manure while Eneji et al., (2002) found 155% of the added N mineralised in urea amended soils. Similar observation was made by Abbasi and Khizar (2012). It is possible that some of the N attributed to the N sources (compost and STSE) originated from the soil organic matter if there was any mineralisation of indegionous organic matter caused by the addition of readily available N (Diaz et al., 2008). From **Table 3-3**, organic C in clay loam was 2.9% as compared to 1.6% in sandy loam. Soil C has been described as one of the major drivers of mineralisation of indigenous organic matter. The readily available N in STSE and the higher soil organic C, likely influenced mineralisation of indigenous organic matter in the clay loam soil. Priming effects have been described as being small, short term and occurring immediately or shortly after addition of a specific substance to the soil (Fontaine et al., 2003; Kuzyakov et al., 2000). In this case, the source of the extra N mineralised from the treatments ($0_{compost}$ +

$37.5_{effluent}$) and ($37.5_{compost}$ + $37.5_{effluent}$) was likely from mineralisation of indigenous organic matter and the recycling of microbial N.

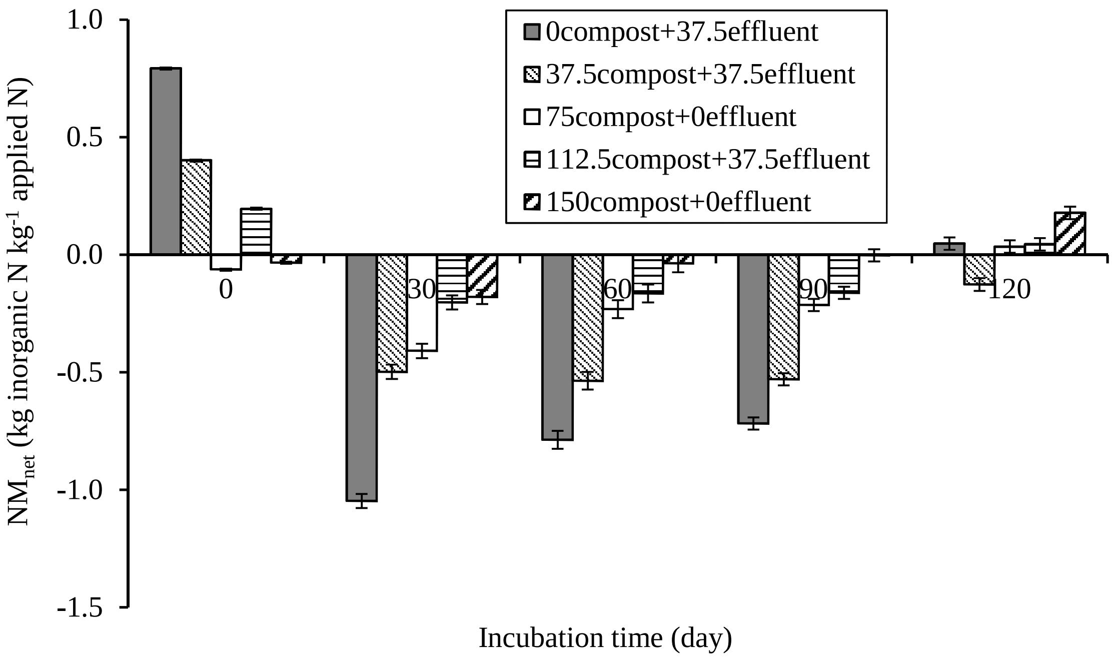


Figure 3-9 Effect of compost, STSE and combinations of compost and STSE amendments on Net N mineralisation (NM_{net}) in the sandy loam soil. ($p = 0.00$). Error bars represent for ± SEM. Negative NM_{net} signifies N immobilisation.

In the sandy loam soil (**Figure 3-9**), the pattern of NM_{net} between 0 and 30 days showed rapid decline for all treatments. The highest decline was observed for the treatment ($0_{compost}$ + $37.5_{effluent}$) to -1.1 kg inorganic N kg^{-1} applied N after 30 days. The decline reduced thereafter up to the end of the incubation. At the start of the incubation, NM_{net} was 0.8, 0.4 and 0.2 kg inorganic N kg^{-1} applied N for the (($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$)) treatments respectively. The presence of compost N in nutrient integration treatments for the sandy loam helped to increase NM_{net} (**Figure 3-9**). Increasing compost contribution as in treatment ($112.5_{compost}$ + $37.5_{effluent}$) resulted in an increase of NM_{net}. On day 30 and 60, NM_{net} was -0.5 and -0.54 kg inorganic N kg^{-1} applied N for the ($37.5_{compost}$ + $37.5_{effluent}$) treatment while for ($112.5_{compost}$ + $37.5_{effluent}$) treatment it was -0.2 and -0.16 kg inorganic N kg^{-1} applied N respectively.

3.3.3 Modelling potentially mineralisable N

The mineral N data was fitted to the one pool model of Stanford and Smith (1972) to determine the influence of the nutrient combinations on the potentially mineralisable N. Potentially mineralisable N (N_o) varies with factors such as moisture, aeration, temperature, nature and amount of organic matter, nature and amount of crop residues left in the soil, and other physical, chemical and biological factors (Cordovil et al., 2005).

Application of the one pool model assumes that there is a mineral N pattern showing an initial rapid N mineralisation followed by a slower linear release of N (Stanford and Smith, 1972). The decline of mineral N (N_{min}) during the first 30 days **(Section 3.3.1)** explained why the mineral N data for treatments in sandy loam soil could not be fitted to the one pool model. The N_{min} data for treatments in sandy loam followed the pattern that was described by Chae and Tabatabae (1986) as showing an initial immobilisation of N, followed by N mineralisation.

The results of model fitting for compost and STSE nutrient integration in clay loam soil are presented in **Figure 3-10**. Increasing the quantity of compost as in treatments ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) resulted in a lower N_o of 124 and 117 mg kg^{-1} respectively. In treatments with STSE ($0_{compost}$ + $37.5_{effluent}$) and compost alone ($75_{compost}$ + $0_{effluent}$), the values of N_o were 127 and 108 mg kg^{-1} respectively (**Figure 3-10**). The differences of potentially mineralisable N between these treatments were not significantly different ($p > 0.05$).

For the treatment ($150_{compost}$ + $0_{effluent}$), the one pool model was only fitted to results of the first 90 days (**Figure 3-10e**). The variability of N dynamics that occurred after 90 days in the treatment with compost alone ($150_{compost}$ + $0_{effluent}$), made model fitting impossible for mineral N data after 90 days. However, the N_0 determined (122 mg kg^{-1}) was higher than for the ($75_{compost}$ + $0_{effluent}$) treatment. Increasing compost application rate in treatments amended with compost alone from 75 to 150 kg N ha^{-1} did not significantly increase the modelled N_0.

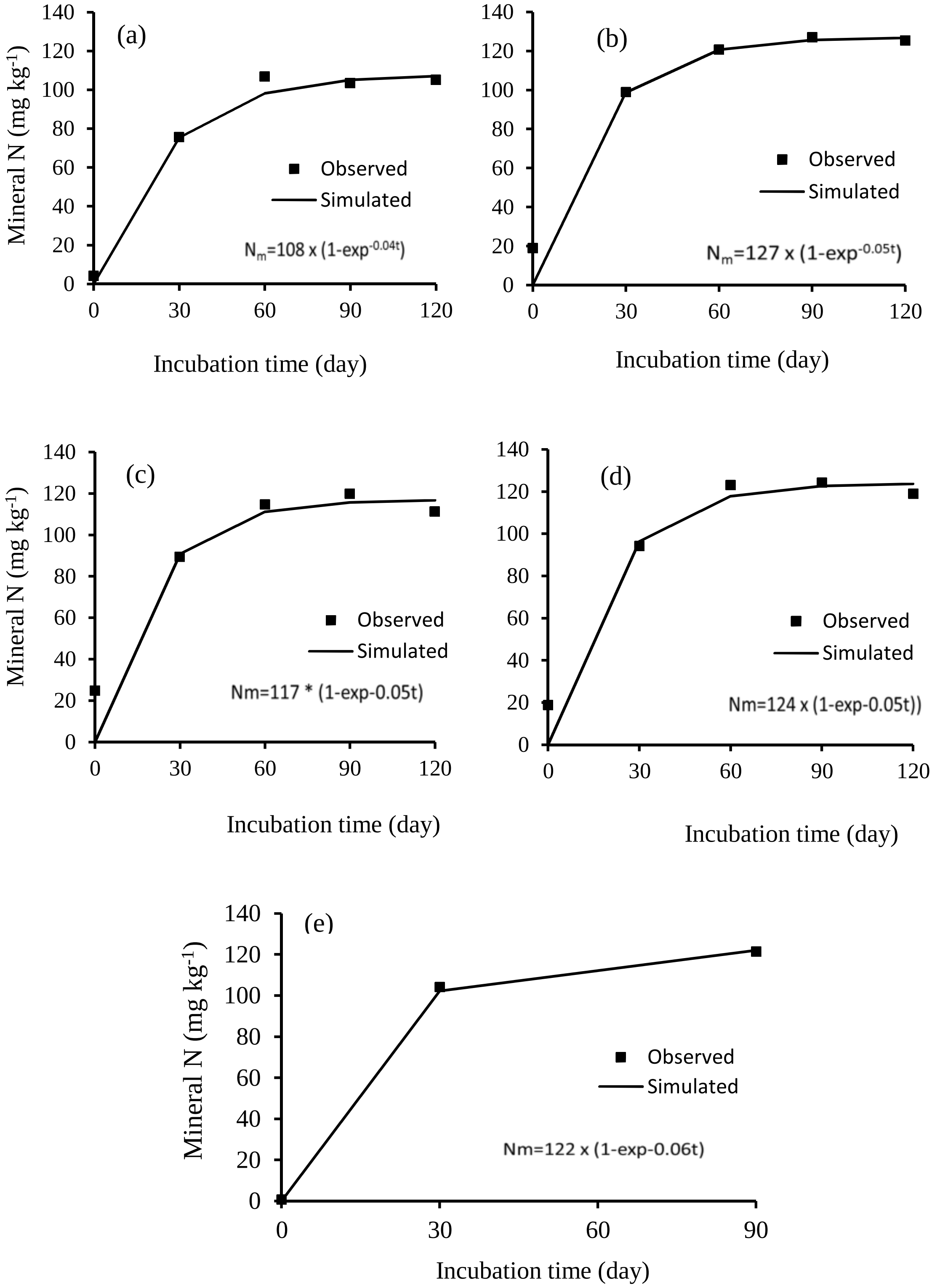


Figure 3-10 Net N mineralisation from treatments in the clay loam soil: (a) 75_{compost} + 0_{effluent}; (b) 0_{compost} + 37.5_{effluent}; (c) 112.5_{compost} + 37.5_{effluent} (d); 37.5_{compost} + 37.5_{effluent} (e) 150_{compost} + 0_{effluent}. Key: ____, model; ▪ observed value. N_m = N_{min} accumulated at time, t.

N mineralisation constants (k) denote the quantity of mineralisable N released per unit time (Serna and Pomares, 1992). In treatments with STSE contribution (($37.5_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$)), k was not significantly different to that from treatments with compost N alone (($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ + $0_{effluent}$)). This showed that for the clay loam soil, the modelled rate of release of potentially mineralisable N was not influenced by the source of N and the combinations of compost and STSE. This was in agreement to Stanford and Smith (1972) as reported by Benbi et al., (2002) who stated that N_0 consists of similar forms of organic N that mineralise at similar rates.

3.3.4 Soil pH, total N, total C and P

3.3.4.1 Soil pH

A general increase of soil pH was observed in both soil types with the largest increment observed for the sandy loam soil. On average, compared to the initial characteristics soil pH increased from 6.8 to 7.8 ($p < 0.05$) in sandy loam while in clay loam soil it was from 7.6 to 8.0 ($p < 0.05$) at the end of the study. As can be observed in **Table 3-3**, initial analysis of soil pH before the start of the incubation study showed that soil pH was higher in the clay loam soil. The interaction of time and soil type was significantly different ($p < 0.05$). However, there were no significant differences between the various combinations of compost and STSE in both soils in relation to soil pH.

The three way interaction of time, soil type and compost- STSE combination with application rates was not significantly different ($p = 0.18$). In sandy loam (**Figure 3-11**), an increasing trend of soil pH was observed for the first 60 days. When averaged across the application rates, time and compost- STSE combinations, the increase in soil pH at the start and end of the incubation in sandy loam was not significantly different ($p = 0.70$). In relation to N availability, Friedel et al., (2000) suggested that an increase in soil pH can also be as a result of N denitrification process that uses up H^+ ions leaving the soil with increased pH. The increase in soil pH in both soils under the various compost and STSE nutrient combinations was not higher enough to affect N mineralisation. The rate of N mineralisation from organic matter is fastest between pH 6 and 8 (Troeh and Thompson, 2005).

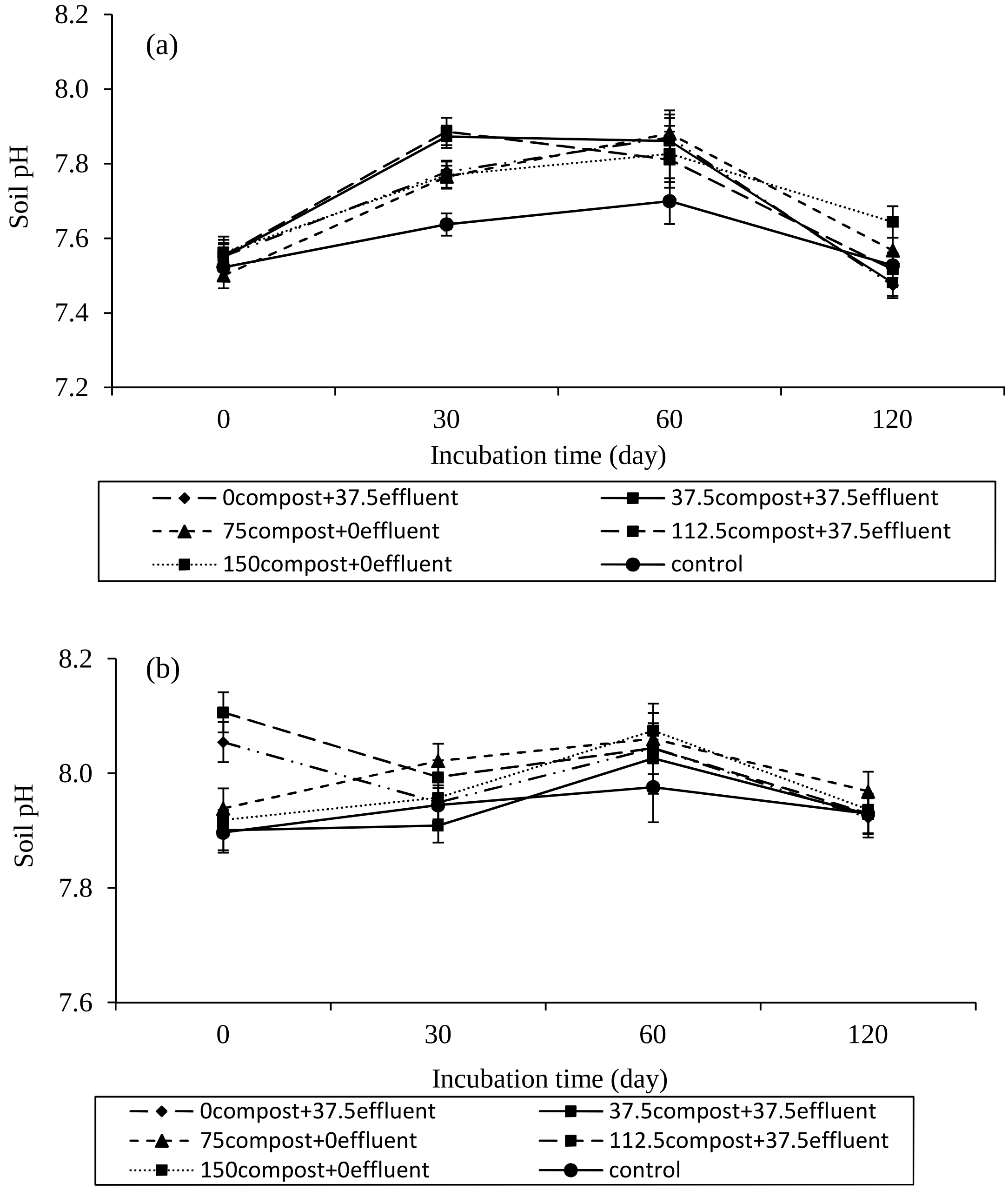


Figure 3-11 Changes of soil pH in a) sandy loam and b) clay loam as result of the combination of compost and STSE. Error bars represent ± SEM and p = 0.18.

3.3.4.2 Soil total N and total C

Analysis of total N sampled at the start and end of the incubation study showed no significant influence of the combinations of compost and STSE on total N ($p = 0.27$). However, a significant treatment effect was observed for the soil types. Total N was significantly higher (p = 0.00) in clay loam soil **(Figure 3-12).** The interaction of soil type and time was also significantly different (p = 0.00).

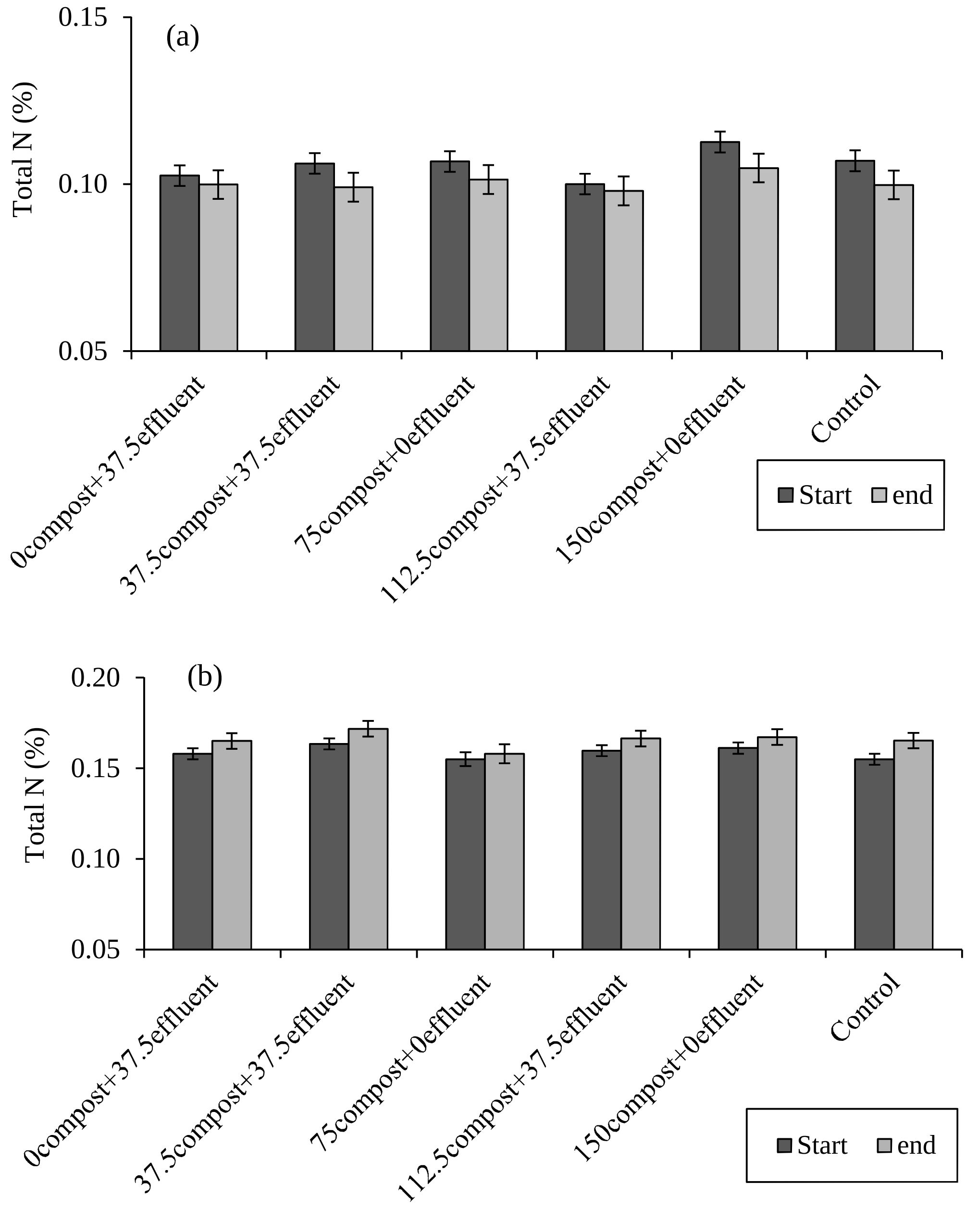
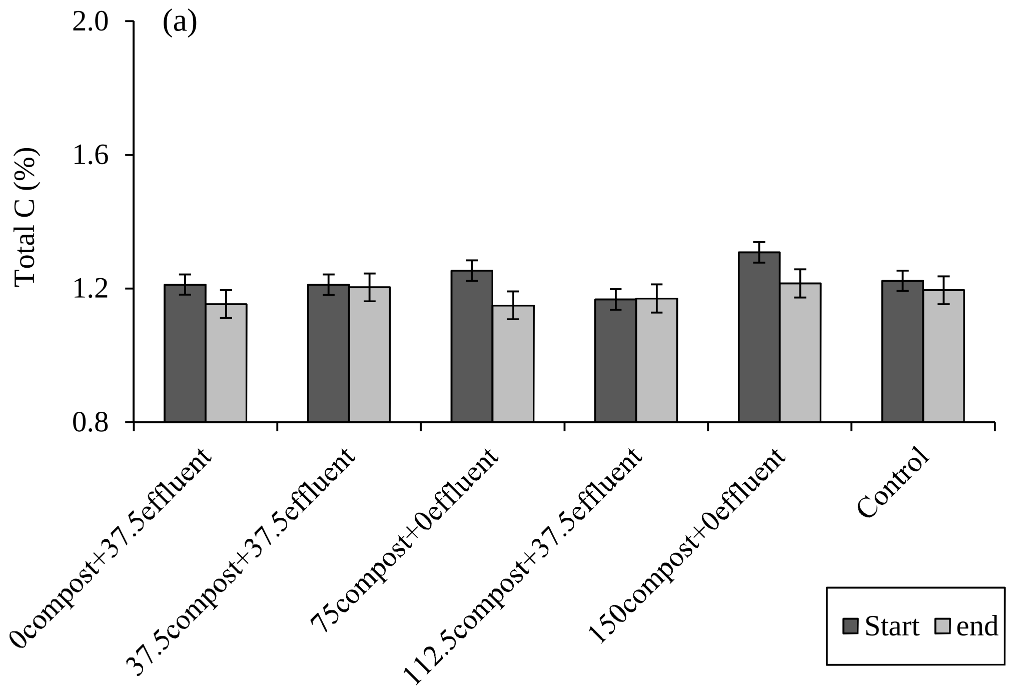


Figure 3-12 Changes of total N in soil in a) sandy loam and b) clay loam as result of the combination of compost and STSE (p = 0.89). Error bars represent ± SEM.

Mean total N in clay loam soil increased (compared to the native soil total N) as a result of the amendment from compost, STSE or the combination of compost and STSE. In clay loam, total N of the native soil was 0.14% and it increased in all the treatments in clay loam to 0.17% ($p < 0.05$).

C availability is an important factor controlling N cycling in soil. Microbial demand for N declines as C availability declines hence more NH_4^+-N becomes available to autotrophic nitrifiers leading to NH_4^+-N conversion to NO_3^--N (Hart et al., 1994). Total C was significantly different with respect to the soil types ($p < 0.05$).



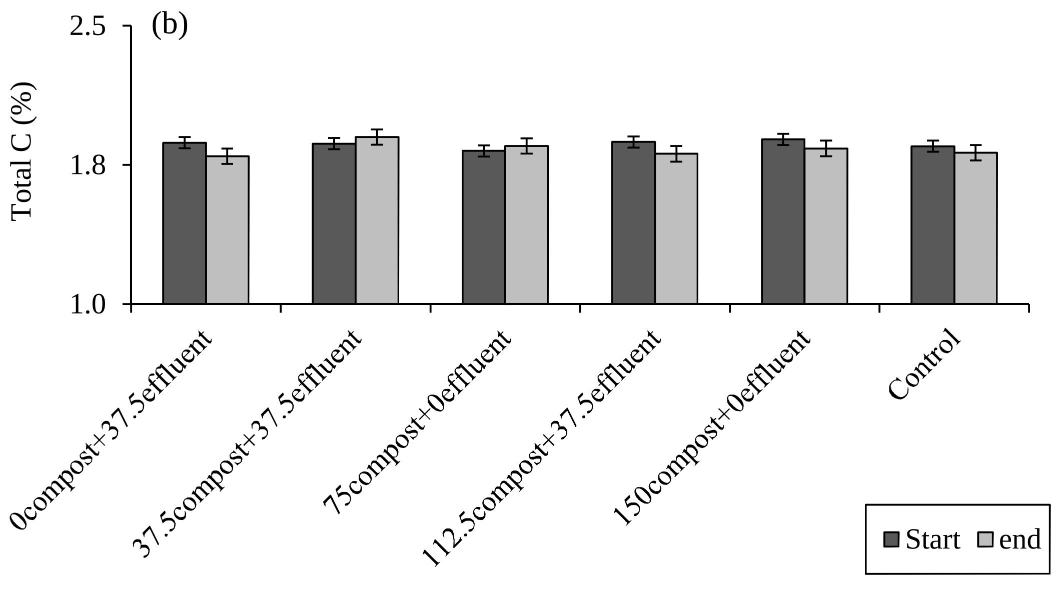


Figure 3-13 Changes of total C in soil in a) sandy loam and b) clay loam as result of the combination of compost and STSE ($p = 0.40$). Error bars represent ± SEM.

Averaged across the compost-STSE combinations, application rates and time; mean total C was significantly higher in clay loam soil (1.84%) as compared to sandy loam soil (1.21%). Compared to the total C of the soils before the incubation study, total C increased slightly in clay loam (0.16%) but at the end of the incubation, a significant reduction of total C was observed in sandy loam (from 1.23% to 1.18%) while a decline of mean total C in clay loam was not significantly different (p = 0.16). The significant decline of total C in sandy loam was from treatments with compost alone (($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ + $0_{effluent}$)) while no significant differences were observed for treatments in clay loam. The combinations of compost and STSE did not significantly influence total C in the soil so was the 3-way interaction of compost-STSE combination with application rate, time and soil type **(Figure 3-13)**.

3.3.4.3 Soil phosphorous

Statistical analysis of total P at the start and end of the incubation study revealed that the combinations of compost and STSE did not significantly influence total P accumulation in the two soils (p = 0.78). However, significant treatment effects were found with respect to the soil types. In the sandy loam, there was a significant increase in total P (p = 0.00) with total P increasing from 938 mg [P] kg^{-1} to 1132 mg [P] kg^{-1} at the end of the study. In the clay loam soil, mean total P decreased significantly ($p < 0.05$) from 998 mg [P] kg^{-1} at the start to 789 mg [P] kg^{-1} at the end of the incubation across all treatments. Overall, mean total P was significantly higher in sandy loam soil as compared to the clay loam. According to Tisdale et al., (1990), P can react with clay minerals to form insoluble phosphate complexes.

Despite the changes that were observed (in clay loam and sandy loam), soil P is known to be strongly insoluble and scarcely mobile in soils with higher pH (Papini et al., 2007). Availability of P is strongly linked to soil pH. Troeh and Thompson (1993) concluded that with soil pH ranging between 6 and 7, availability of total P is not affected. Considering that the soil pH for the clay loam (7.6) was slightly above the limit of Troeh and Thompson (1993), availability of total P was likely affected through retention/fixation.

Similarly, soil extractable P was significantly higher in treatments in sandy loam soil. In sandy loam, the mean extractable P after 120 days did not change as compared to that in the soil before the incubation (40 mg [P] kg^{-1}) while in the clay loam, extractable P increased ($p > 0.05$) from 22 mg [P] kg^{-1} in the soil before the experiment to 27 mg [P] kg^{-1} at the end of 120 days.

Irrespective of the application rates, the combinations of compost and STSE did not significantly influence soil extractable P at the end of the incubation in both soil types. In sandy loam (**Figure 3-14**), extractable P was 38, 39, 41, 40 and 43 mg [P] kg^{-1} for the ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$), ($112.5_{compost}$ +$37.5_{effluent}$), ($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ +$0_{effluent}$) treatments respectively. In clay loam, it was 27, 26, 29, 26 and 28 mg [P] kg^{-1} for the ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$), ($112.5_{compost}$ +$37.5_{effluent}$), ($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ +$0_{effluent}$) treatments respectively.

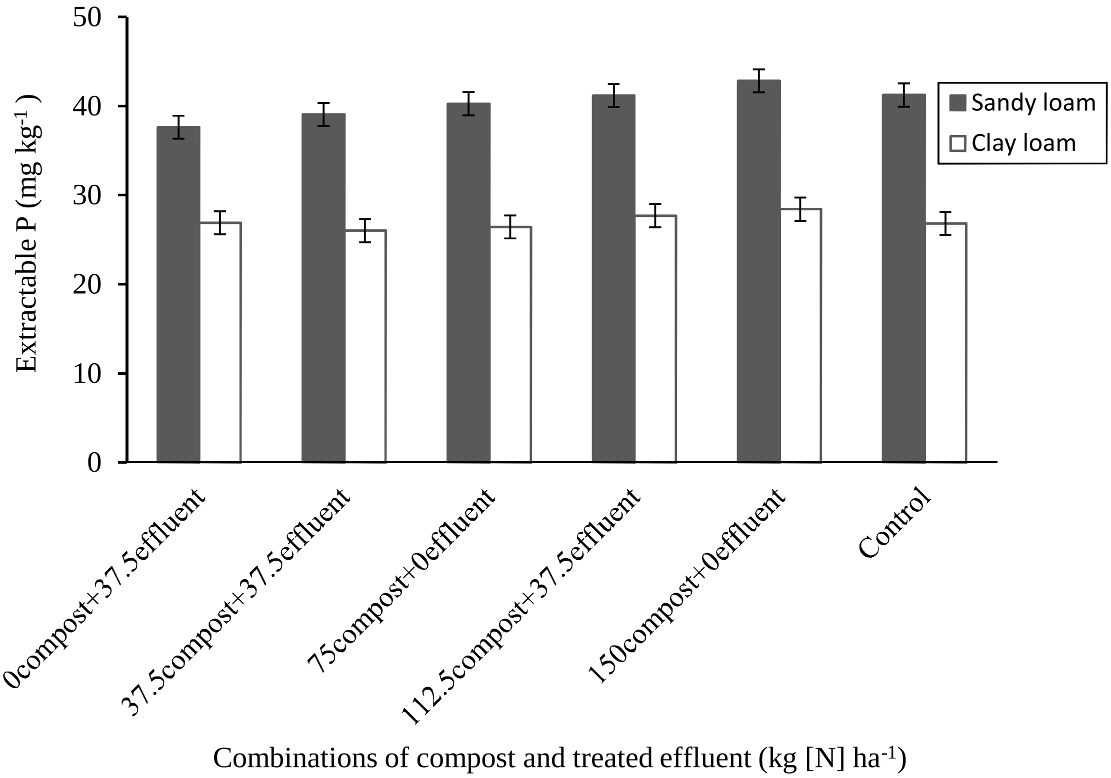


Figure 3-14 Soil extractable P at the end of the incubation study following the application of compost, STSE and a combination of compost and STSE ($p = 0.73$). Error bars represent ± SEM.

In the absence of plant uptake, excess P in the soil solution and the readily available pool is likely to move to the less readily available and the very slowly available pools (Antille, 2011). Unfortunately in these pools extractable P cannot be measured by standard soil analyses protocols. When water-soluble P (fertiliser, STSE, etc.) is added to soil, a very small proportion remains in the soil solution and a small part may undergo initial precipitation reactions in some soils (Syers et al., 2008). However, the majority of the P rapidly becomes distributed between the readily-available and less readily available pools by processes of adsorption and then absorption. These processes can give an insight to extractable P dynamics mainly in treatments with STSE contribution (($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ +$37.5_{effluent}$).

3.3.5 Microbial biomass

Microbial biomass study was conducted to further understand and link N release patterns observed for mineral N in the clay loam and sandy loam to microbial biomass C and N. From the trends reported and discussed in **Section 3.3.2**, it was expected that MBN would be significantly higher (N immobilisation) in treatments with STSE alone and combinations compost and STSE in sandy loam. In the clay loam, MBN was expected to be low due to the N release (N mineralisation) that has been reported in **Section 3.3.2**. However, variability of the data resulted in inconclusive results for MBN.

3.3.5.1 Soil microbial biomass carbon

Microbial biomass C was significantly higher ($p = 0.00$) in clay loam soil as compared with the sandy loam soil. The mean MBC was 137 and 86 mg kg^{-1} for clay loam and sandy loam soils respectively. The interaction of soil type, combinations of compost and STSE, application rates and time was not significantly different ($p = 0.86$). In the sandy loam, mean MBC for the various combinations of compost and STSE was not significantly different ($p > 0.05$). Treatments with STSE and combinations of compost and STSE, showed higher MBC at the start before declining. An increase was later observed after 30 days (**Table 3-8**). This trend was similar to N dynamics reported in **Section 3.3.2.**

Table 3-8 MBC dynamics in the sandy loam soil. Mean MBC (mg kg^{-1}) with different letters are significantly different (P < 0.05). Numbers in parenthesis are ± SEM.

Compost and STSE combinations	MBC (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}$+$37.5_{effluent}$	143(46)	62(11)	101(14)	102(12)	87(19)	99^{a}
$37.5_{compost}$+$37.5_{effluent}$	90(56)	79(14)	81(17)	93(15)	69(24)	82^{a}
$75_{compost}$+$0_{effluent}$	53(46)	70(11)	106(14)	81(12)	86(19)	79^{a}
$112.5_{compost}$+$37.5_{effluent}$	136(46)	64(11)	75(14)	70(12)	75(19)	84^{a}
$150_{compost}$+$0_{effluent}$	106(46)	95(11)	89(14)	65(12)	84(19)	88^{a}
Control	103(46)	65(11)	92(14)	86(12)	85(19)	86^{a}

In clay loam (**Table 3-9**), mean MBC during the incubation period was lower in the control and in treatments ($150_{compost}$+$0_{effluent}$) and ($112.5_{compost}$+$37.5_{effluent}$). MBC declined with time for treatments with STSE and combinations of compost and STSE (($0_{compost}$+$37.5_{effluent}$), ($37.5_{compost}$+$37.5_{effluent}$) and ($112.5_{compost}$+$37.5_{effluent}$)). Comparing treatments with STSE and combinations of compost and STSE, MBC was in the order ($0_{compost}$+$37.5_{effluent}$) > ($37.5_{compost}$+$37.5_{effluent}$) > ($112.5_{compost}$+$37.5_{effluent}$). But mean MBC for these treatments was not significantly different.

Table 3-9 MBC dynamics in the clay loam soil. Mean MBC (mg kg^{-1}) with different letters are significantly different (P < 0.05). Numbers in parenthesis are ± SEM.

Compost-STSE Combinations	MBC (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}$+$37.5_{effluent}$	256(79)	163(19)	177(24)	120(21)	125(34)	168^{a}
$37.5_{compost}$+$37.5_{effluent}$	232(46)	108(11)	152(14)	132(12)	126(19)	150^{ab}
$75_{compost}$+$0_{effluent}$	134(56)	162(13)	147(17)	158(15)	174(24)	155^{ab}
$112.5_{compost}$+$37.5_{effluent}$	160(46)	106(11)	154(14)	125(12)	95(19)	128^{bc}
$150_{compost}$+$0_{effluent}$	105(46)	142(11)	156(14)	101(12)	130(19)	127^{bc}
Control	90(56)	92(13)	126(17)	99(15)	127(24)	107^{c}

Energy supply is the most important limiting factor affecting microbial activity (Troeh and Thompson, 2005). For most microbes the energy supply consists of animal and plant residuals. Compost and STSE supplied microbial energy through organic matter and dissolved organic matter respectively. Lack of significant differences for mean MBC in combinations of compost and STSE in the sandy loam soil indicated that the level of energy supply available for microbes was the same.

3.3.5.2 Soil microbial biomass nitrogen

MBN during the incubation study was not significantly influenced by the soil types ($p = 0.48$). But the three way interaction of soil type, compost-STSE combination with application rate and incubation time was significantly different ($p = 0.03$). When aggregated across the soil types, application rates and the compost- STSE combinations in sandy loam, MBN was significantly different ($p = 0.00$) with respect to the incubation time. Higher MBN was observed from day 60 to the end of the incubation study. The highest MBN was observed on day 60 of 59.8 mg kg^{-1}. As shown in **Table 3-10** and **Table 3-11**, mean MBN for treatments in sandy loam and clay loam were not significantly different to each other and in both soils, the combinations of compost and STSE did not influence MBN ($p = 0.83$).

Table 3-10 MBN dynamics in the sandy loam soil. Mean MBN (mg kg^{-1}) with different letters are significantly different ($P < 0.05$). Numbers in parenthesis are ± SEM.

Compost-STSE Combinations	MBN (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}$+$37.5_{effluent}$	2.9(2)	7.9(3)	3.5(23)	5.5(10)	4.4(9)	4.8^{a}
$37.5_{compost}$+$37.5_{effluent}$	2.4(2)	0.2(3)	11.5(23)	7.3(10)	0.2(9)	4.3^{a}
$75_{compost}$+$0_{effluent}$	0.8(2)	0.2(3)	59.8(23)	20.5(10)	6.7(9)	17.6^{a}
$112.5_{compost}$+$37.5_{effluent}$	1.7(2)	1.5(3)	9.8(23)	42.6(10)	0.2(9)	11.1^{a}
$150_{compost}$+$0_{effluent}$	2.4(2)	0.2(3)	0.2(23)	18.4(10)	26.5(9)	9.5^{a}
Control	1.7(2)	0.2(3)	37.2(23)	3.2(10)	9.6(9)	10.4^{a}

Table 3-11 MBN dynamics in the clay loam soil. Mean MBN (mg kg^{-1}) with different letters are significantly different ($P < 0.05$). Numbers in parenthesis are ± SEM.

Compost-STSE Combinations	MBN (mg kg^{-1})					
	0	30	60	90	120	Mean
$0_{compost}+37.5_{effluent}$	0.2(4)	0.2(6)	70.9(39)	0.2(17)	0.2(15)	14.3^{a}
$37.5_{compost}+37.5_{effluent}$	2.4(2)	7.2(3)	0.2(23)	19.0(10)	20.1(9)	9.8^{a}
$75_{compost}+0_{effluent}$	4.7(3)	1.5(4)	30.6(28)	0.2(12)	58.5(11)	19.1^{a}
$112.5_{compost}+37.5_{effluent}$	9.0(2)	5.6(3)	3.8(23)	0.2(10)	14.3(9)	6.6^{a}
$150_{compost}+0_{effluent}$	5.7(3)	0.2(4)	52.5(28)	0.2(12)	0.2(11)	11.7^{a}
Control	4.6(2)	0.2(3)	12.7(23)	0.2(10)	7.6(9)	5.0^{a}

Soil microbial biomass as an active component of the terrestrial ecosystem regulates functions and processes related to soil (Wright and Islam, 2005). Some of these processes and function include source-sink in nutrient cycling, decomposition of organic residue, structural stability and indicator of soil pollution and bioremediation. Soil microbial biomass activity and accumulation in soil is related to several factors, for example soil texture, soil moisture, organic C and N limitations and temperature. Moisture condition is a major factor controlling survival and activity of microorganisms in the soil (Zhang et al., 2005). Adequate soil moisture increases microbial biomass and activity. Beyond field capacity, microbial activity decreases with increasing moisture, due to limited oxygen availability. Rosacker and Kieft (1990) reported that microbial biomass increased when grassland soil was moistened to between 50 and 60% water holding capacity; but microbial biomass declined greatly as the soils were subjected to progressive drying conditions. This incubation study was set at a moisture content of 100 and 98% of water holding capacity for treatments in sandy loam and clay loam soils respectively.

The results of MBN have not provided clear explanations to the trend of mineral N and N mineralisation that was observed in **Section 3.3.2**. This could be as a result of a number of reasons including spatial variations of microbial biomass within the sampling space or limitations of the fumigation-extraction method that was used to extract soil samples. Chloroform fumigation works well in soils with lower moisture content in which case chloroform is uniformly distributed in the soil sample. In soil samples at higher moisture content, biomass may not be exposed to the fumigant leading to underestimates (Azam et al., 2003).

3.4 Conclusion

The main conclusions from the incubation experiments are summarised below:

a) N dynamics and release of mineral N has been influenced by the soil types, as such the response to compost and STSE nutrient integration on N dynamics was soil specific.
   - Release of NO_3^--N was significantly higher in treatments in clay loam while in the sandy loam, N immobilisation affected release of NO_3^--N especially in treatments with STSE-N contribution (($0_{compost}$+$37.5_{effluent}$) and $37.5_{compost}$+$37.5_{effluent}$)).
   - Mineral N was significantly higher in treatments in the clay loam soil. Mean mineral N in the clay loam was 95 mg kg^{-1} while in the sandy loam it was 19 mg kg^{-1}.

b) For the clay loam soil, net N mineralisation was significantly higher in treatments with STSE. Increasing the quantity of compost in compost and STSE nutrient integration resulted in reduced net N mineralisation in the clay loam. In the sandy loam, increasing compost contribution in a treatment combination of compost and STSE resulted in a significant increase of net N mineralisation. On day 30 and 60, for the treatment ($37.5_{compost}$ + $37.5_{effluent}$), NM_{net} was -0.5 and -0.54 kg inorganic N kg^{-1} applied N while for treatment ($112.5_{compost}$ + $37.5_{effluent}$), it was -0.2 and -0.16 kg inorganic N kg^{-1} applied N respectively.

c) N mineralisation in treatments with STSE alone and combination of compost and STSE was higher than the applied N. On day 60, net N mineralisation was 2.5 and 1.2 kg inorganic N kg^{-1} applied N for the ($0_{compost}$ + $37.5_{effluent}$) and ($37.5_{compost}$ + $37.5_{effluent}$) treatments respectively.

d) In both soil types, combinations of compost and STSE did not significantly influence total N, total C and P. However, these were influenced by the soils types used in the incubation study.

e) Microbial biomass N and C in both soils did not provide any conclusive results to support the results of N dynamics and mineralisation. This was due to the huge variability of the data as a result of the method and moisture content that was used for the incubation.

The results obtained in this chapter are essential to the subsequent experimental chapters (Pot/Glasshouse and lysimeter experiments) in terms of providing explanations and understanding with regards to nutrient availability, leaching and ryegrass yield production.

4 GLASSHOUSE STUDY

This chapter presents and discusses the glasshouse (pot) experiment that was carried out from April 2010 to April 2012. The glasshouse (pot) experiment was conducted to determine the impact of repeated application of compost and STSE on crop production and soil properties. As presented in **Chapter 1**, the glasshouse/pot study contributed towards meeting objectives I, II and IV of the research study about mechanism of interaction, ryegrass production and optimal combinations of compost and STSE. Pots were set in a glasshouse facility and perennial ryegrass *(Lolium perenne)* was grown. The soils in the pots were amended with greenwaste compost and irrigated with STSE to supply 75 or 150 kg N ha^{-1} according to the various combinations of compost and STSE that were developed.

4.1 Introduction

The pot experiment was undertaken from April 2010 for a duration of two years. The experiment was set in a semi-controlled environment to establish the long term role in terms of nutrient provision and the impact on soil chemical properties as a result of STSE irrigation on soils amended with compost while contributing towards the main objectives of the research outlined in **Chapter 1**.

The experiment was set to compliment the results from the lysimeter experiment (**Chapter 5**). The main aim of the study was to determine the effects of repeated application of STSE and compost on ryegrass production and soil properties.

The specific objectives of the glasshouse experiment are presented below;

i. To determine the response of ryegrass dry matter (DM) production to the nutrient supply combinations of compost and STSE.
ii. To determine the impact of the nutrient supply combinations of compost and STSE on the biological, chemical and physical properties of soil.
iii. To quantify the response of ryegrass N uptake and N use efficiency (NUE) as influenced by the nutrient supply combinations of compost and STSE.

4.2 Materials and methods

4.2.1 Description of pot experiment

The pot experiment was conducted in a glasshouse facility at Cranfield University (**Figure B-1**). The soils used were the sandy loam and clay loam. The soils and greenwaste compost used were of the same batch as those used in the incubation study (**Chapter 3**). The soils were selected as they had distinctive chemical and physical characteristics. Soil texture was verified by analysing the soil samples using the pipette method (Avery and Bascomb, 1982; BSI, 1990).

The pot experiment involved the use of triplicate samples in a randomised complete block design of two soil types: sandy loam and clay loam; five combinations of compost and STSE: ($0_{compost}$ + $100_{effluent}$), ($25_{compost}$ + 75_{STSE}), ($50_{compost}$ + $50_{effluent}$), ($75_{compost}$ + $25_{effluent}$) and ($100_{compost}$ + $0_{effluent}$) and two N application rates: 75 and 150 kg ha^{-1} which resulted in a total of 60 pots. The combinations of compost and STSE were developed on a percentage basis. Soil samples were air-dried and ground to pass through a 2 mm mesh screen. Using a bulk density of 1400 kg m^{-3} for both soils, 6.3 kg of soil was packed in 10 l pots on top of a layer of gravel (500 g) at the bottom of the pot (to prevent soil loss and allow free drainage). In case of leaching, the collected leachate was poured back on to the pots. The quantity of compost applied per pot was calculated on a volumetric basis assuming a soil depth of 0.15 m and a bulk density of 1400 kg m^{-3}.

Perennial ryegrass *(Lolium perenne)* was grown in the pots. Ryegrass was chosen as it allows for monitoring of N uptake and DM yield regularly after harvesting (cutting). A summary of the combinations of compost and treated effluent developed and the quantities of compost and effluent applied has been presented in **Table 4-1**. The quantity of STSE applied depended on the total N in effluent. During preparation of the pots, greenwaste compost was mixed with the soil in pots and subsequently, ryegrass seeds were evenly spread at a rate of 4 g (seeds) m^{-2} (Antille, 2011). The seeds were covered with a thin soil layer to facilitate germination. To avoid disturbing the seeds and splashing the soil during the germination period, water bottles were used to spray water to the pots until after germination. Sowing was conducted on 20th April 2010 and germination was recorded approximately 10 days later. STSE irrigation started on 18th May 2010. Irrigation with STSE started after ryegrass germination because of the higher

NH_4^+ concentration in the treated effluent at that particular time that would have affected germination of the seeds.

Table 4-1 Quantity of greenwaste compost and STSE applied to corresponding nutrient supply combinations to provide 75 or 150 kg N ha^{-1} to ryegrass plants.

N application rate (kg ha^{-1})	Treatment combinations (%)	Compost application rate		Effluent (ml pot^{-1})	
		(ton ha^{-1})	(g pot^{-1})	2010/11	2011/12
75	$0_{compost} + 100_{effluent}$	0	0	4688	4090
	$25_{compost} + 75_{effluent}$	1.13	3.8	3073	3521
	$50_{compost} + 50_{effluent}$	2.3	7.8	2314	2020
	$75_{compost} + 25_{effluent}$	3.41	11.4	1169	1020
	$100_{compost} + 0_{effluent}$	4.53	15.1	0	0
150	$0_{compost} + 100_{effluent}$	0	0	12500	8182
	$25_{compost} + 75_{effluent}$	2.3	7.8	9336	6111
	$50_{compost} + 50_{effluent}$	4.57	15.2	6212	4060
	$75_{compost} + 25_{effluent}$	6.83	22.8	3103	2030
	$100_{compost} + 0_{effluent}$	9.1	30.3	0	0

At the start of the second year (February 2011), a second cycle of the glasshouse experiment was initiated by simulating soil tillage and applying compost. Tillage whilst ryegrass plants were still growing was a huge restriction to compost application. A second effluent irrigation cycle was also initiated. A pictorial overview of the pot experiment in the glasshouse is shown in **Figure 4-1**.

Figure 4-1 A pictorial overview of the Glasshouse (pot) experiment showing a) establishment of ryegrass in the pots, b) growth stage of the grass and c) ryegrass cutting.

4.2.2 Measurement and analysis

4.2.2.1 Soil analysis

Before the pot experiment, the soils (Sandy loam and Clay loam) and the greenwaste compost were analysed to establish background physical and chemical characteristics of the two soils and the greenwaste compost. The results have been reported in **Section 3.3.1** of **Chapter 3**. At the start of the experiment (after amending with appropriate quantities of greenwaste compost), day zero soil sampling and analysis was conducted to establish initial background reference values. **Table 4-2** provides a summary of the analyses conducted and their frequency during the 2-year pot experiment. During soil sampling, an auger (15 mm diameter) was used to collect soil samples that were homogenised through mixing. The holes left after sampling where backfilled with similar corresponding soil.

NO_3^--N and NH_4^+-N was determined using the *Burkard Scientific* Segmented Flow Analyser. Sample extraction was done on 20 g fresh soil; 100 ml of 2 mol l^{-1} potassium chloride (KCl) solution was used to extract the sample before filtration using Whatman No.4 filter paper (MAFF, 1986a). Soil pH was determined in 1:5 soil/water extracts (BSI, 2000c) while in STSE, pH and electrical conductivity (EC) were measured using *Jenway 4400* pH and conductivity meter.

Measurements of TN and TC in soil and compost were made at the start and end of the study on fine ground dried samples by catalytic tube combustion using the *Vario EL III*, *CHNOS elemental analyser* (BSI, 2000b). Soil samples were acid–digested using the aqua regia process for the determination of total phosphorous in soil (TP_{soil}) using spectrophotometry (*Nicolet Evolution 100*) (BS EN 13657, 2002). Determination of plant extractable P in soil was conducted using sodium hydrogen carbonate (BSI, 1995). Soil organic matter was determined on dehydrated air dried soil by loss-on-ignition (BSI, 2000a). Cation exchange capacity (CEC) of the soil was determined using barium chloride (BSI 7755., 1996).

Heavy metals were analysed in soil samples at the start of the study, at the end of the first year (2011) and the second year (2012). Copper (Cu), Zinc (Zn), Lead (Pb), Chromium (Cr) and Nickel (Ni) were analysed in the pot study. Measurement of heavy metals was done using the Atomic Absorption Spectrophotometer (*AAnalyst 800*) on soil samples digested using the aqua regia technique (US EPA, 1994).

Table 4-2 Details of soil and plant analyses conducted during the two years of the pot experiment in the glasshouse (April 2010 to April 2012).

Determination	Timing and frequency of analysis
TN_{soil}	• Start of study, end of 1st year and end of 2nd year
TC_{soil}	• Start of study, end of 1st year and end of 2nd year
Heavy metals (Cr, Cu, Pb, Ni & Zn)	• Start of study, end of 1st year and end of 2nd year
Extractable P	• End of first year and end of 2nd year
TOC	• Start of study, end of 1st year and end of 2nd year
Potassium	• Start of study, end of 1st year and end of 2nd year
Total P	• Start of study, end of 1st year and end of 2nd year
Organic matter	• After each ryegrass cut (1st year) • Start and end of second year
TN_{plant}	• After each ryegrass cut for both years
Dry matter (above ground)	• After each ryegrass cut for both years
Mineral N	• After each ryegrass cut (1st year) • Start and end of second year
pH	• After each ryegrass cut (1st year) • Start and end of second year
Air temperature	• Continuously from 2011 to 2012
$TN_{effluent}$	• After each effluent irrigation event
NH_4^+-$N_{effluent}$	• After each effluent irrigation event
NO_3^--$N_{effluent}$	• After each effluent irrigation event
$P_{effluent}$	• Twice a month during effluent irrigation season
$K_{effluent}$	• Twice a month during effluent irrigation season
Conductivity	• After each effluent irrigation event
$pH_{effluent}$	• After each effluent irrigation event
Orthophosphate $_{effluent}$	• After each effluent irrigation event

4.2.2.2 Plant analysis

In the first year, three ryegrass cuts were made compared to five cuts in the second year. The plant herbage was harvested by cutting at about 2 cm above the soil surface (Cordovil et al., 2006). Harvested plant material was oven-dried at 60˚C for 48 hours (Evers, 2002). Measurement of TN_{plant} was made on fine ground dried samples by catalytic tube combustion using the *Vario EL III*, *CHNOS* elemental analyser (BSI, 2000b). Ryegrass N uptake was determined as the product of TN_{plant} and ryegrass dry matter (DM). Total phosphorous in plants (TP_{plant}) was determined using the aqua regia method for acid digestion of the plant material and with spectrophotometry technique (*Nicolet Evolution 100*) for determination of TP_{plant} (US EPA, 1994).

4.2.3 Secondary treated sewage effluent irrigation

Irrigation was done manually using STSE in treatments with combination of compost and STSE. Pots were irrigated with STSE until the quantities reported in **Section 4.2.1** were attained. In treatments with effluent contribution (($0_{compost}$ + $100_{effluent}$), ($25_{compost}$ + $75_{effluent}$), ($50_{compost}$ + $50_{effluent}$) and ($75_{compost}$ + $25_{effluent}$)), upon attaining the effluent volumes mentioned in **Table 4-1**, the pots were irrigated with deionised water. In treatments with no effluent nutrient contribution e.g. ($100_{compost}$ + $0_{effluent}$), pots were irrigated with deionised water from the onset.

Determination of the amount of effluent or deionised water to irrigate was based on estimates of evapotranspiration readings taken from an ETgage™ that was installed in the glasshouse. ETgage™ (**Figure 4-2**) is a modified atmometer and is a convenient, practical tool for irrigation management. An atmometer measures evaporation from a wet porous surface, commonly a ceramic disk (Hess, 1996). It provides useful information which can be used to accurately estimate the quantity of water to apply and schedule irrigation. Since the ETgage™ was used in a controlled environment, a coefficient of correlation (1.6) was determined and used to correlate and calibrate the ETgage™ to properly estimate crop evapotranspiration (ET_c) in the glasshouse facility. It was determined by relating ETgage™ measurements to reductions in mass from the pots which was as result of water loss through evapotranspiration.

Figure 4-2 ETgage used to determine evapotranspiration loss of water from the pots in the glasshouse for the determination of amount of deionised water/STSE to irrigate.

Total N in treated effluent ($TN_{effluent}$) was analysed immediately while the other analyses were done within 5 days of effluent collection. All effluent samples were stored at 5ºC pending analysis. Analysis of $TN_{effluent}$ was done immediately to monitor the quantity of N applied in treatments with effluent N contribution. STSE was regularly analysed for total N, NH_4^+ -N, NO_3^- -N, P, K, pH, conductivity and Orthophosphate. Nutrient characterisation of the STSE was done by employing reactive kits using Spectroquant Merck® test kits as described in **Section 3.2.3** (**Chapter 3**). The concentrations of dissolved Cu, Zn, Ni, Cr and Pb in STSE were measured using an Atomic Absorption Spectrophotometer (AAnalysist 800) on effluent samples filtered through 0.45 µm filters. Chemical characteristics of the STSE have been presented in the **Section 4.3**, **Table B.1-6** and **Table B.1-7** in Appendices**.**

4.2.4 Statistical analysis

The effect of each treatment and the influence of soil type, application rates and compost-effluent combinations on the measured variables within and between the two years of the pot experiment were assessed by repeated measures analysis of ANOVA (General Linear Models) in Statistica 9.0 to determine significant difference of means. Significantly different levels of treatments were identified using least significant differences at a probability of 0.05 (Fisher's LSD). Probability plots of residuals were used to assess whether or not a data set was approximately normally distributed. Occasionally, extreme values were removed during the statistical analyses.

4.3 Results and discussion

4.3.1 Soil, compost and STSE characteristics

The sewage effluent used had undergone secondary treatment to satisfy EU regulations for discharge quality. Using the FAO classification (Ayers and Westcot, 1985) reported in **Chapter 2** (Literature review), the STSE was classified as having none to slight to moderate restriction for agricultural use. Hence it could be used for agricultural purposes without causing negative environment impacts. For effluent samples in both years, the STSE was almost neutral with mean pH of 6.8 and 6.7 for 2010/11 and 2011/12 respectively.

The results of the analyses of STSE are presented in **Table 4-3**. Total N in the STSE was predominantly in the form of mineral N (NH_4^+-N and NO_3^--N) with calculated dissolved organic N making about 12% of total N in the first year. In the second year, dissolved organic N was 16% of total N. $TN_{effluent}$ increased from 36 to 55 mg l^{-1} in the second year. This increase in $TN_{effluent}$ resulted in reduced quantity of effluent required for the respective treatments (**Table 4-1)**. The increase of $TN_{effluent}$ in the second year also resulted in an increment of NO_3^--N to 40 mg l^{-1}.

Initial analysis of STSE showed low to non-detectable levels of heavy metals in the STSE. Mean dissolved Pb, Cu and Cr was 0.08 (±0.03), 0.005 (±0) and 0.016 (±0.006) mg l^{-1} respectively. Ni and Zn were non-detectable in the STSE. As reported in **Chapter 3** (**Section 3.2.3**), CUSTP is a smaller treatment plant processing sewage from a residential catchment. As such the concentrations of heavy metals in the STSE were low. Hence as compared to other treatment works, e.g. Anglian Water, Severn Trent/Southern Water, despite that all treatment plants meets the EU directive on wastewater, the concentration of heavy metals was likely lower at CUSTP.

Table 4-3 Chemical and physical properties of STSE during the glasshouse (pot) experiment with standard error of the means (SEM).

Parameter	2010/11	SEM	2011/12	SEM
K (mg l^{-1})	22	1.43	22	1.48
TN (mg l^{-1})	36	1.85	55	3.02
NH_4^+ - N (mg l^{-1})	4	0.45	2.7	0.67
NO_3^--N (mg l^{-1})	27	1.32	40	1.85
P (mg l^{-1})	5.7	0.34	6.8	0.34
Conductivity (µS cm^{-1})	764	18.23	855	22.25
pH	6.8	0.17	6.7	0.23
*DOC (mg l^{-1})			88	6.15

*DOC is dissolved organic carbon

Table 4-4 Chemical and physical properties of compost and soil prior to the start of the pot experiment. Numbers in parenthesis are ± SEM.

Parameter	Sandy loam	Clay loam	Compost
Cu (mg kg^{-1})	15 (0.7)	16 (0.6)	4 (0.3)
Cr (mg kg^{-1})	32 (5.0)	22 (2.0)	10 (2.2)
Ni (mg kg^{-1})	17 (4.0)	17 (1.0)	UD**
Pb (mg kg^{-1})	79 (10.2)	22 (8.4)	8 (2.0)
Zn (mg kg^{-1})	59 (8.9)	80 (9.6)	31 (2.8)
K (mg kg^{-1})	6 (0.7)	16 (0.8)	
CEC (cmol+ kg^{-1})	10 (0.15)	17 (0.18)	

UD undetectable**

Table 4-4 presents additional characteristics of the greenwaste compost and soils used in the pot study. Selected characteristics of compost, clay loam and sandy loam soil used for the pot study were already presented in Chapter 3. The concentration of heavy metals in greenwaste compost was below PAS 100 limits (BSI, 2005) and was below the maximum permissible concentration of potential toxic elements (MAFF, 1998). According to BSI (2005), the upper limits for Cu, Pb, Cr, Zn and Ni are 200, 200, 100, 400 and 50 mg kg^{-1} respectively, however Smith (2009) identified Pb as the most limiting element to compost usage in domestic gardens.

4.3.2 Ryegrass production

4.3.2.1 Ryegrass dry matter

As previously stated, a total of three cuts were made in 2010/11 (first year) and five ryegrass cuts in the second year (2011/12). The time difference between the cuts for the two years was variable as it was governed by the growth characteristics of the ryegrass. Ryegrass was harvested as soon as three leaves had fully developed per tiller (EBLEX., 2013). Allowing a fourth leaf resulted in loss of dry matter yield (DM) as the old leaves started to die off. In the first year, the ryegrass cutting intervals were 55, 91 and 268 days after germination for the three cuts while in the second year, they were 85, 130,177, 288 and 449 days after germination for the five cuts made. Cutting time interval was significantly influenced by the weather patterns. Longer cutting intervals were associated with the winter season (cold) while shorter intervals were in spring and summer. Morrison et al., (1980) suggested fixing cutting interval of grass for studies of similar nature, but with variations in weather, the risk of not having sufficient herbage to harvest becomes higher. This is why the cutting intervals in this study were governed by the actual growth of the ryegrass in the glasshouse. Anslow and Green (1967) adopted an approach of less frequently cutting grass in autumn and winter season to allow for adequate grass growth before cutting.

In the first year compost was applied and mixed with soil within the top 10 cm soil depth and irrigation with sewage effluent only started after ryegrass establishment. In the second year, soil tillage was undertaken on the top 5 cm soil depth and green waste compost was incorporated. The extent of compost incorporation in the soil was affected by the growing ryegrass in the pots. In the second year, immediately after applying compost, STSE irrigation commenced.

The results of DM yield analyses have been presented in **Figure 4-3**, **Figure 4-4**, **Figure 4-5** and **Figure 4-6**. DM yield corresponding to the individual cuts for each of the two-year study period are presented in Appendix (**Figure B-2** and **Figure B-3**).

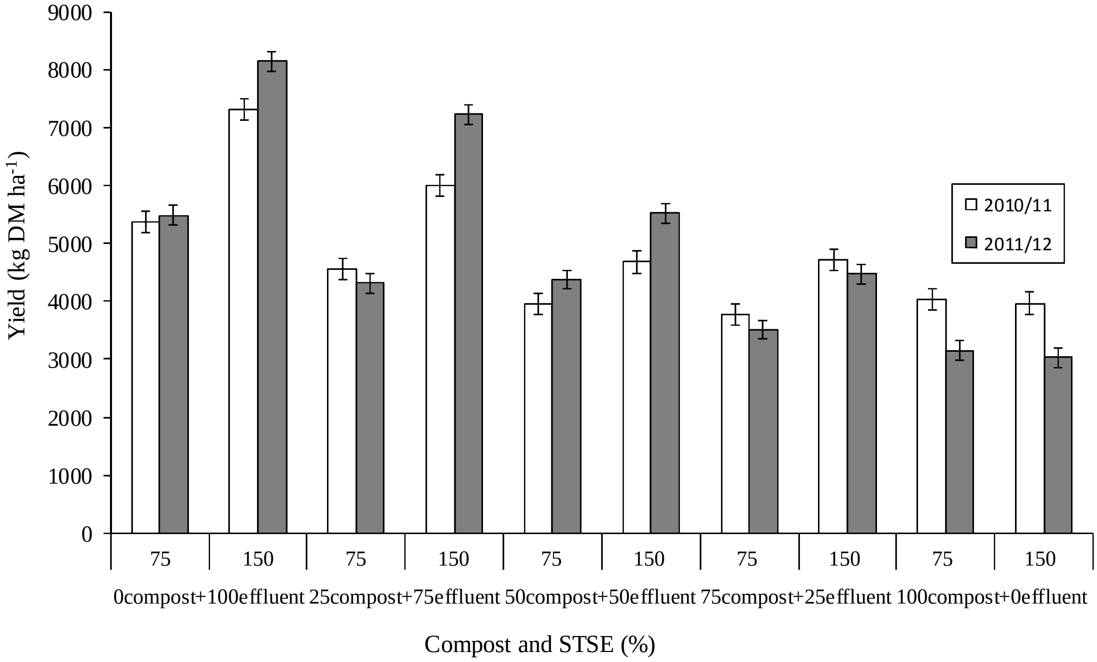


Figure 4-3 Mean yearly dry matter yield for the various combinations of compost and STSE supplying either 75 or 150 kg N ha^{-1} in sandy loam for 2010/11 and 2011/12 seasons (p = 0.036). Error bars represent ± SEM.

The response of the interaction of combinations of compost and STSE, soil type and application rate on dry matter yield is presented in **Figure 4-3** and **Figure 4-4** for 2010/11 and 2011/12 respectively. Statistical analysis of mean total DM yield for the first year, showed that DM was significantly influenced ($P < 0.05$) by the soil type, application rates and the combinations of compost and sewage effluent. In the first year (2010/11), increasing N application rates from 75 to 150 kg total N ha^{-1} significantly increased mean DM yield from 5685 to 6808 kg DM ha^{-1}. A study by Anslow and Green (1967) reported by Morrison at al., (1980) found that perennial ryegrass has a characteristic pattern of seasonal rate of growth even when supplied with adequate N and water throughout the growing season. The interaction of environmental factors and ryegrass growth can have a significant influence on DM yield within the growing seasons.

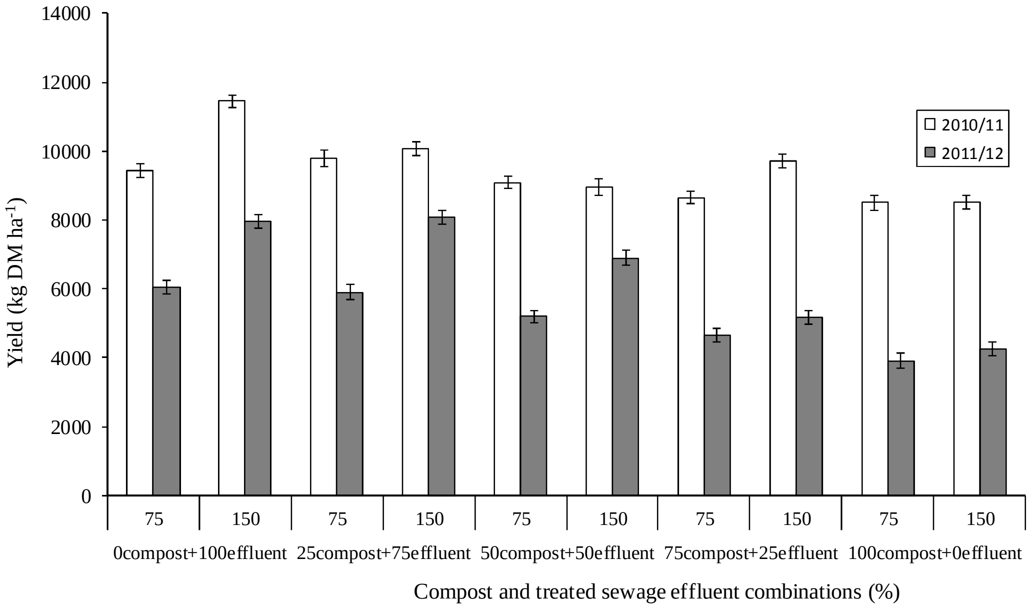


Figure 4-4 Mean yearly dry matter yield for the various combinations of compost and STSE supplying either 75 or 150 kg N ha^{-1} in clay loam for 2010/11 and 2011/12 seasons ($p = 0.036$). Error bars represent SEM

The four way interaction of time, soil type, application rates and combinations of compost and STSE was significantly different ($p = 0.036$). DM yield production (for the two soil types, N application rates and compost-STSE combinations) declined with time. Mean DM yield (for the two soil types, N application rates and compost-STSE combinations) declined from 7127 kg ha^{-1} in the first year to 5366 kg ha^{-1} in the second year. However, analysis of DM yield for the individual soils for the duration of the experiment showed that the decline was largely associated with the clay loam soil. DM yield in clay loam soil declined by *c.* 62% while in sandy loam between the years, overall increase of DM yield was 2%. However, in both years DM yield of ryegrass was significantly higher in the clay loam soil as compared to sandy loam. As reported in **Chapter 3**, N mineralisation and availability of N was significantly higher in the clay loam soil. Low N mineralisation in sandy loam affected availability of inorganic N for dry matter production.

For the 2010/11 in the sandy loam soil, higher DM yield ($p < 0.05$) was harvested from the treatment ($0_{compost}$+$100_{effluent}$) at N application rate of 150 kg total N ha^{-1} of 7321 kg ha^{-1} (**Figure 4-3**). Addition of compost in the combinations of compost and STSE e.g.

($25_{compost}$+$75_{effluent}$) treatment, DM yield significantly reduced to 6008 kg ha^{-1}. DM reduced significantly further for the treatment ($50_{compost}$+$50_{effluent}$) to 4680 kg ha^{-1}. A similar trend was observed for the same year but at N application rate of 75 kg total N ha^{-1}. At 75 kg total N ha^{-1} in sandy loam, DM yields were 5377, 4556, 3957, 3768 and 4036 kg DM ha^{-1} for the treatments ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$), ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively.

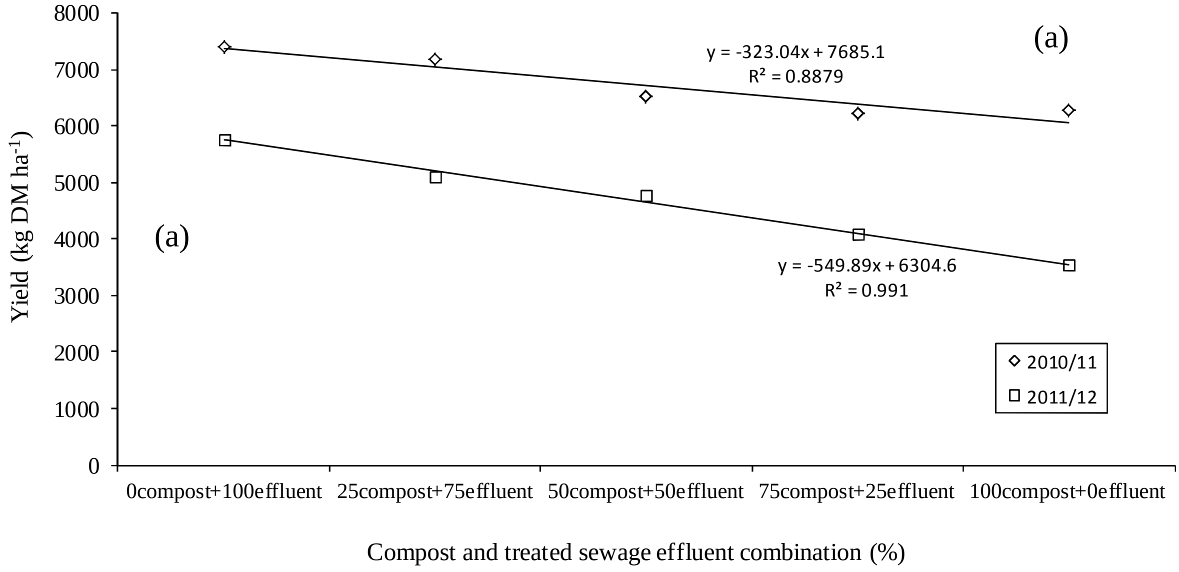
In the clay loam soil (**Figure 4-4)**, in the first year DM was significantly higher ($p < 0.05$) in treatment with effluent N alone (($0_{compost}$+$100_{effluent}$)) than the treatment with 25% N contribution from compost (($25_{compost}$+$75_{effluent}$)). This trend was similar to what was observed for the sandy loam for the same treatments in both years. DM yield decreased with increasing compost contribution. However, in the second year, no significant differences in DM were observed. Otherwise for the rest of the combinations of compost and STSE, DM decreased with increasing compost contribution as has been outlined earlier on. In **Chapter 3**, an observation was made that availability of mineral N was dependent on the proportion of compost in combined application of compost and STSE such that N availability was affected with increasing proportion of compost.

Air temperature in the glasshouse was recorded hourly using Type EC95 thermistors (temperature range 0 to 70 ± 0.1°C) from Dec 2010 to April 2012. The results have been presented in **Appendix (Figure B-4)**. Earlier studies in the same facility (2007-09) by Antille (2011) showed that air temperatures inside the glasshouse can be on average, up to 60% higher than the outside air temperature. To prevent soil freezing in winter, air temperature in the glasshouse was maintained at a minimum of 10°C but sensitivity of net N mineralisation is maximal at 25°C (Guntiñas et al., 2012). Anslow and Green (1967) reported a relationship between ryegrass growth and temperature that showed optimum temperature of 15 – 18°C. An increase in temperature may improve the ability of shoots to expand leaf surface (Beadle, 1997). Ryegrass cuts made just after the winter season had low DM yield. Ryegrass cuts made in summer and winter responded accordingly to the higher air temperature in the glasshouse. Availability of N to plants was enhanced amongst others with increased temperature. The source of N to the ryegrass plants was from several sources, N mineralised from soil organic matter, compost and/or effluent N and free living soil organisms (Morrison et al., 1980).

The interaction between time, N application rates and combinations of compost and effluent were significantly different (p = 0.04) and has been presented with linear equations in **Figure 4-5**. At all N application rates, the combinations of compost and treated effluent were linearly related to each other and to DM yield. DM yield reduced with increasing contribution of compost in combined application of compost and STSE.

Irrespective of N application rates, the relationship between the combinations of compost and STSE and soil type was significantly different ($p < 0.05$). The mathematical function fitted on the data showed the linearity of the relationship between the soil types and the combinations of compost and sewage effluent on DM yield. In both soil types, DM yield reduced with increasing contribution of compost in a compost-effluent combination.

Slope of the mathematical function fitted (linear equation) indicate the rate of decrease of DM yield with increasing compost contribution in compost-STSE nutrient combinations. In **Figure 4-5a** the rate of DM yield decrease at N application rate of 75 kg N ha^{-1} with increasing compost contribution was higher in the second year (2011/12 season). At N application rate of 75 kg N ha^{-1}, the rate of decline for every addition of compost in a combination of compost and STSE was *c.*550 kg DM ha^{-1} as compared to *c.*323 kg DM ha^{-1} for the second year. Similarly at 150 kg N ha^{-1} (**Figure 4-5b**), the rate of decline of DM yield was higher in 2011/12 (*c.*1166 kg DM ha^{-1}) as compared to the first year (713 kg DM ha^{-1}).



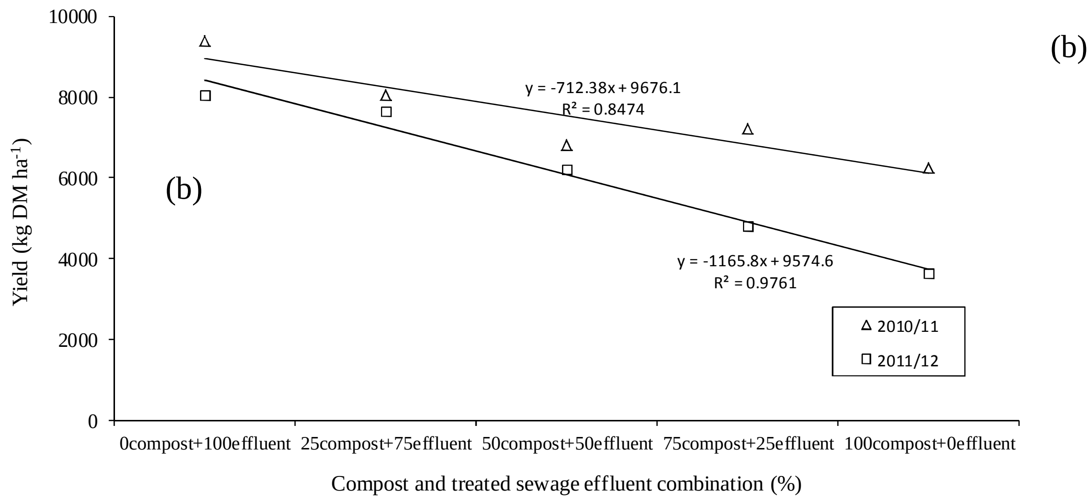
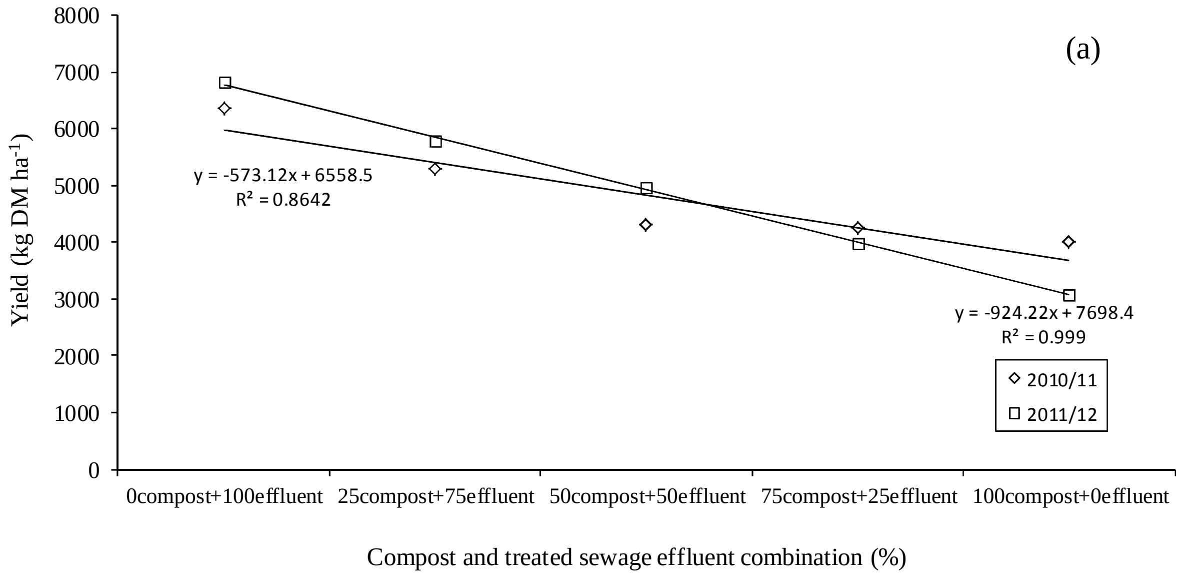


Figure 4-5 Relationship between combinations of compost and STSE and dry matter yield for the sandy loam and the clay loam soils (a) 75 kg N ha^{-1} and (b) 150 kg N ha^{-1} for 2010/11 and 2011/12 seasons (p = 0.00).

In relation to soil type (**Figure 4-6**), the rate of DM yield decline for the combinations of compost and STSE for a unit increase in compost contribution in both years was higher in the sandy loam ($P < 0.05$). In 2010/11, for every unit addition of compost in a combination of compost and STSE, DM yield declined by *c.*573 and *c.*462 kg DM ha^{-1} in sandy loam and clay loam respectively. In the second year, similarly the rate of decline for a unit increase in compost contribution in the compost-effluent combinations was higher in sandy loam (924 DM ha^{-1}) than in clay loam soil (791 kg DM ha^{-1}).



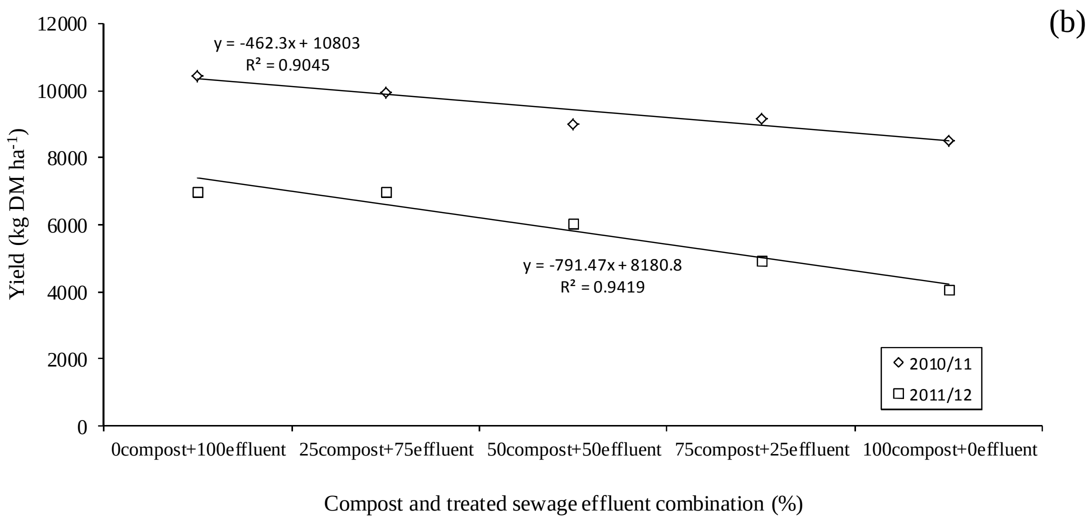


Figure 4-6 Relationship between compost and STSE combinations and ryegrass dry matter yield for 75 and 150 kg N ha^{-1} (a) sandy loam and (b) clay loam (p = 0.00).

4.3.2.2 Nitrogen in harvested plant material

TN_{plant} plays a significant role in many essential functions and processes in plants. N forms part of chlorophyll in plants and it is responsible for capturing light energy for production of sugars for the plant. The results for TN_{plant} are shown in **Figure 4-7 and Figure 4-8** for the two N application rates. Statistically, TN_{plant} was significantly influenced by the soil types, application rates and the combinations of compost and STSE. TN_{plant} was significantly higher ($p < 0.05$) at an N application rate of 150 kg total N ha^{-1} as compared to 75 kg total N ha^{-1}. Increasing N application rate from 75 to 150 kg total N ha^{-1} significantly increased mean TN_{plant} from 1.7% to 1.8% (w w^{-1}). In relation to the soil types, TN_{plant} increased significantly by 25% in the clay loam.

Overall, significant differences were observed due to the combinations of compost and STSE. Increasing the contribution of compost in a combination of compost and STSE reduced TN_{plant}. TN_{plant} decreased in the order ($0_{compost}$+$100_{effluent}$) < ($25_{compost}$+$75_{effluent}$) < ($50_{compost}$+$50_{effluent}$) < ($75_{compost}$+$25_{effluent}$) < ($100_{compost}$+$0_{effluent}$). However, TN_{plant} for the treatments ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$) were not significantly different ($p = 0.58$).

TN_{plant} decreased significantly in between the years. In 2010/11 and 2011/12, mean TN_{plant} decreased from 1.8% to 1.6% (w w^{-1}) representing a decrease of 14%. This decrease was largely from treatments in clay loam soil **(Figure 4-7b and Figure 4-8b)**. With time, the interaction of soil type, application rates and the combination of compost and treated effluent did not significantly influence TN_{plant} ($p = 0.48$). This relationship has been presented in **Figure 4-7 and Figure 4-8.**

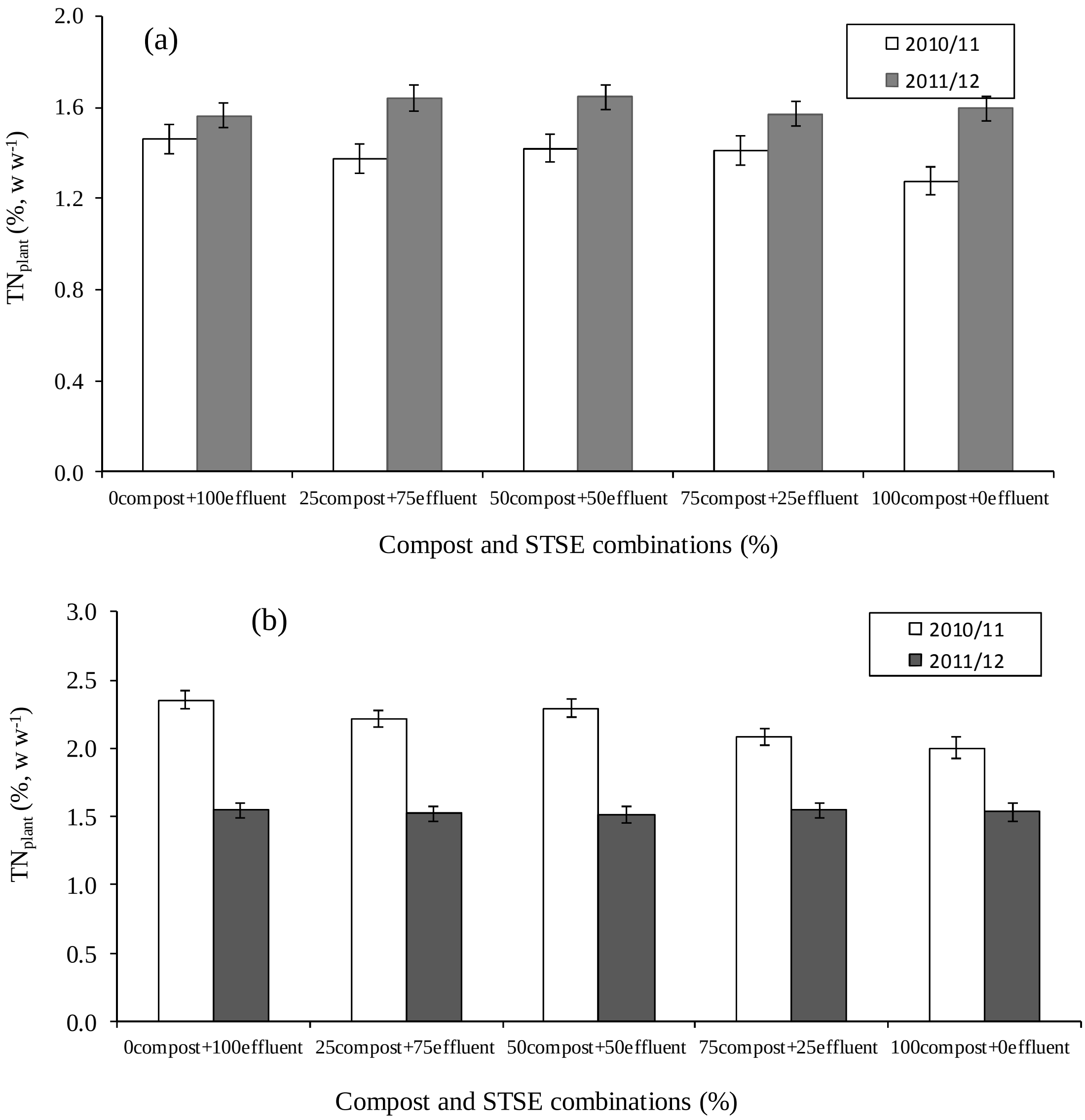
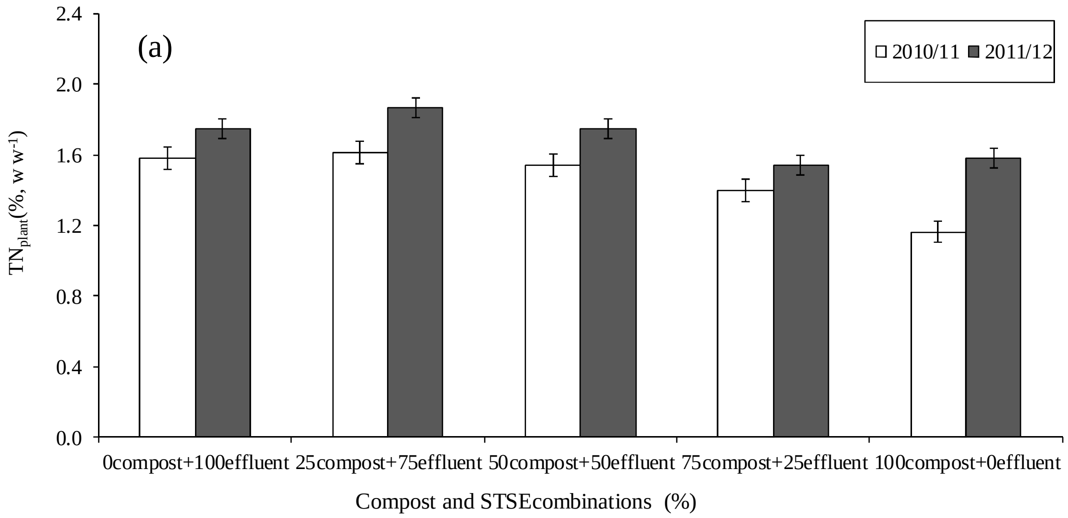


Figure 4-7 Mean TN_{plant} for the various combinations of compost and STSE supplying 75 kg total N ha^{-1} in (a) sandy loam and (b) clay loam for 2010/11 and 2011/12 seasons (p = 0.48). Error bars represent ± SEM.

It is important to note that the decline in DM yield observed in the combinations of compost and STSE in the clay loam soil at both N application rates in the second year (**Figure 4-4**) corresponded with TN_{plant} decline in **Figure 4-7b and Figure 4-8b**. Reduced TN_{plant} in the second year resulted into a decline of DM yield in the clay loam soil. In the sandy loam, this relationship was not much clear especially at N application rate of 75 kg total N ha^{-1}. However at N application rate of 150 kg total N ha^{-1} in the sandy loam soil, an increase in TN_{plant} was related to an increase of ryegrass DM yield for the treatments ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$).



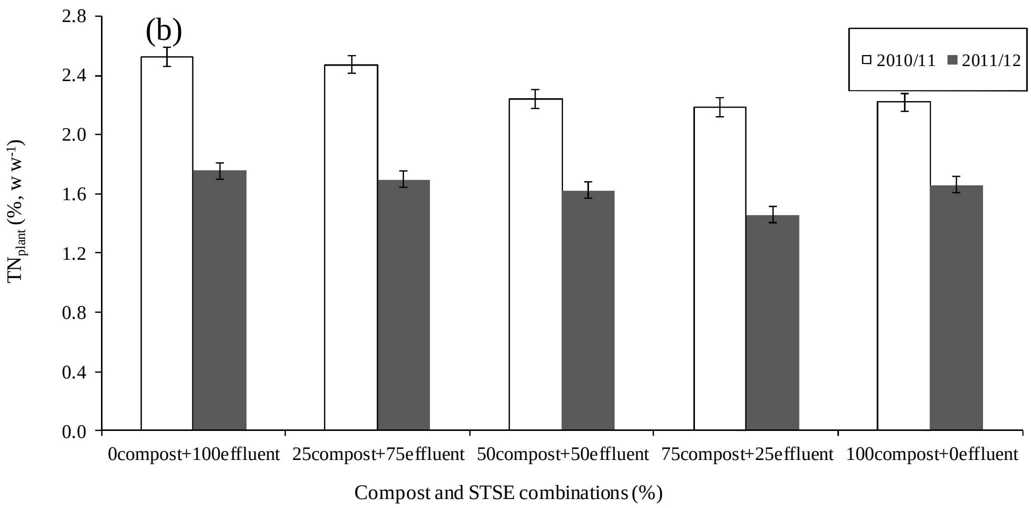


Figure 4-8 Mean TN_{plant} for the various combinations of compost and STSE supplying 150 kg N ha^{-1} in (a) sandy loam and (b) clay loam for 2010/11 and 2011/12 seasons ($p = 0.48$). Error bars represent ± SEM.

Figure 4-9 shows the relationship between DM yield and TN_{plant} for both soils and also the TN_{plant} limits for beef and dairy cattle. In both soils, the correlation between DM $_{yield}$ and TN_{plant} decreased over time. TN_{plant} with time increased in the sandy loam soil. In **Chapter 3** for the sandy loam, it was reported that N immobilisation reduced with time thereby increasing availability of N. That could explain why TN_{plant} increased with time in the sandy loam soil.

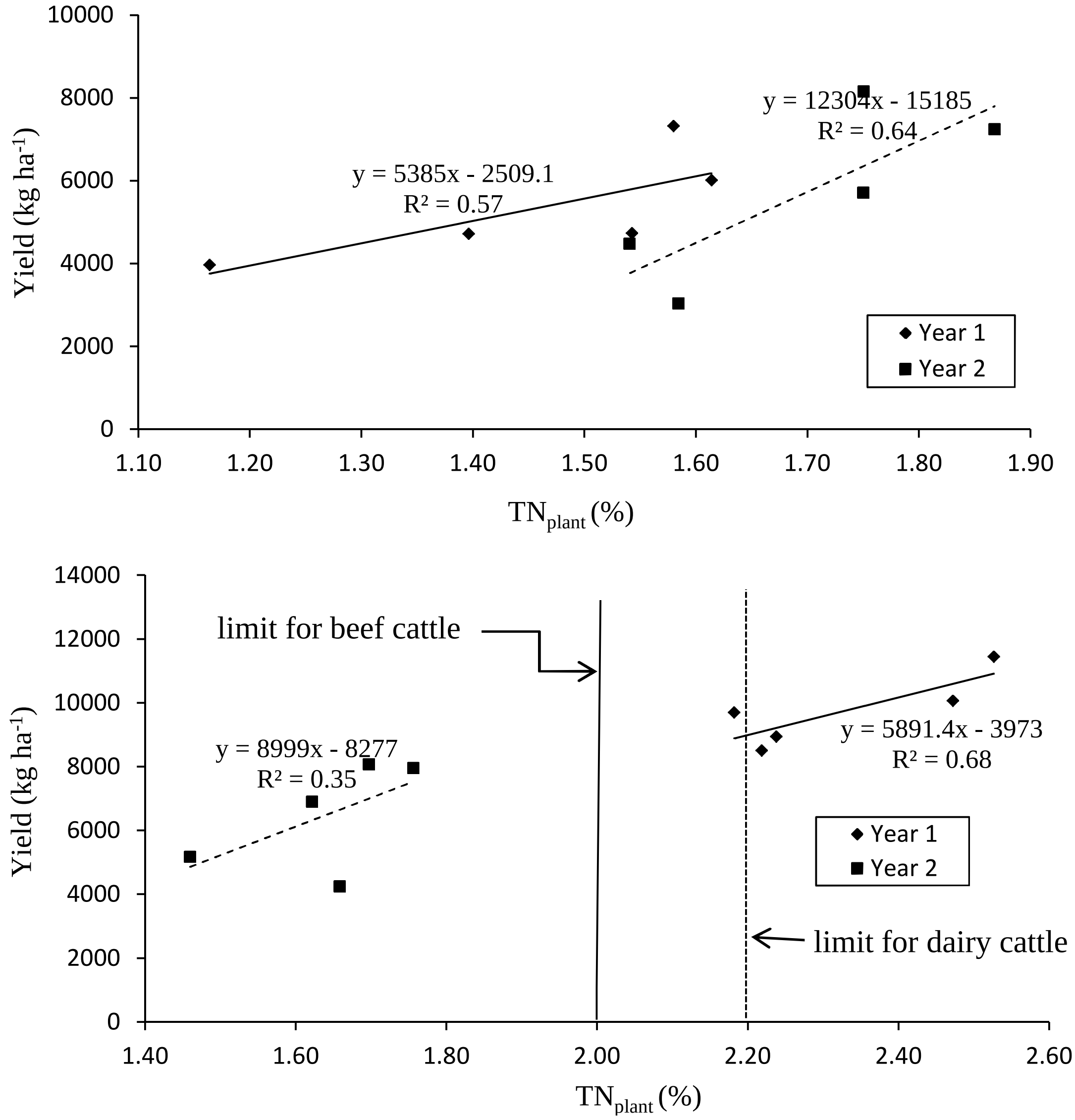
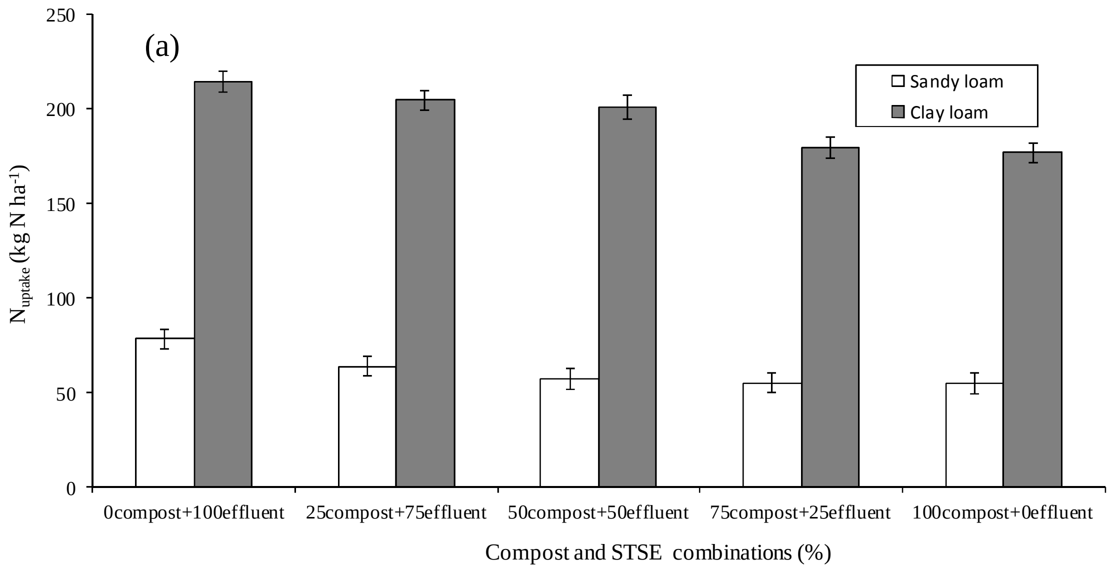


Figure 4-9 Relationship between ryegrass DM_{yield} and TNplant for the first and second year of the pot experiment in a) sandy loam and b) clay loam soil.

4.3.2.3 Nitrogen plant uptake

Nitrogen uptake (N_{uptake}) was estimated after each and every ryegrass cut in both years. It was estimated as a product of TN_{plant} and DM yield (Douglas et al., 2003; Brink et al., 2001). This approach was successfully used by Kokorra (2008) and Antille (2011) to estimate N uptake from organic amendments. Total N uptake was estimated by summing up N_{upake} from individual ryegrass cuts. N uptake has been used previously as an indicator of N dynamics (N mineralisation and immobilisation) by Chadwick et al., (2000). The estimated N uptake has been presented in **Figure 4-10** and **Figure 4-11.**



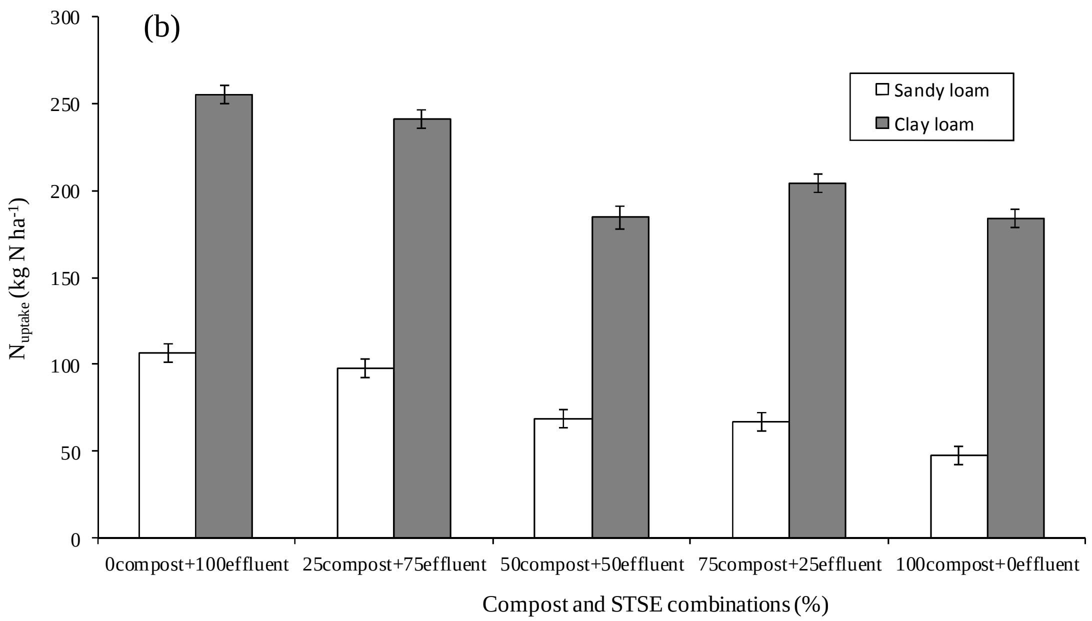
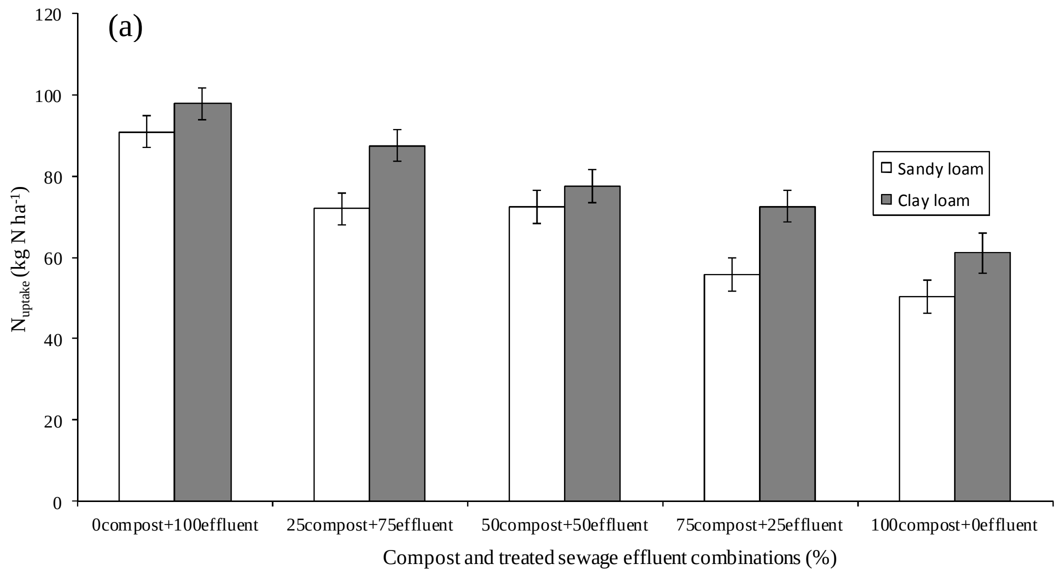


Figure 4-10 Total nitrogen uptake for the combinations of compost and STSE in the sandy loam and the clay loam in 2010/11 at N application rates of (a) 75 kg N ha^{-1} and (b) 150 kg N ha^{-1} ($p = 0.01$). Error bars represent ± SEM.



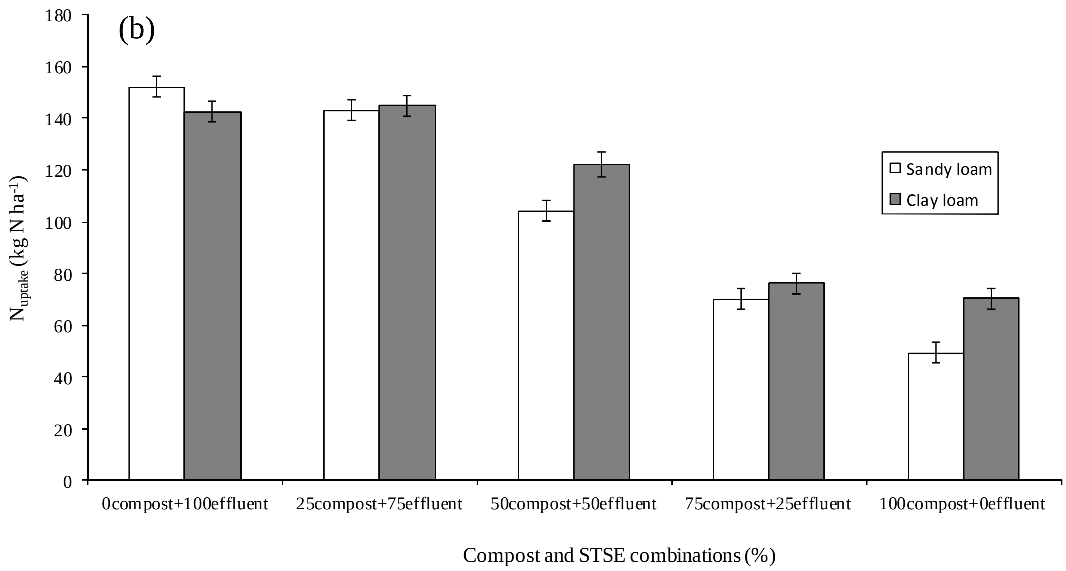


Figure 4-11 Total nitrogen uptake for the combinations of compost and STSE in sandy loam and clay loam in 2011/12 at N application rates of (a) 75 kg N ha^{-1} and (b) 150 kg N ha^{-1} ($p = 0.01$). Error bars represent ± SEM.

Analysis of total N_{uptake} for 2010/11 and 2011/12 showed that N uptake was significantly influenced by soil type, N application rates and the combinations of compost and STSE. The mean N_{uptake} in sandy loam (*c*.78 kg ha^{-1}) was significantly lower ($p < 0.05$) as compared to N_{uptake} in clay loam soil (*c*. 150 kg ha^{-1}). Initial analysis of the soil before the start of the experiment reported in **Chapter 3** showed that clay loam soil was much more fertile as compared to sandy loam. The initial organic C in the clay loam was 2.9% as compared to 1.6% in the sandy loam. TN_{soil} in the clay loam was 0.14% while in sandy loam, it was 0.12%. Clay loam was also higher in organic matter (4.8%) as compared to the sandy loam soil (3.7%).

N_{uptake} assessed between the years was significantly affected by the interaction of N application rate, soil type and combinations of compost and STSE (**Figure 4-10** and **Figure 4-11**). In the clay loam and sandy loam soils at both N application rates, N_{uptake} was significantly affected by N application rates. A similar observation can be made in **Figure 4-11a** for 2011/12 at the N application rate of 75 kg ha^{-1}.

Irrespective of the soil types and N application rates, the combinations of compost and STSE significantly influenced total N_{uptake}. For the duration of the glasshouse/pot experiment, mean N_{uptake} was significantly higher for the ($0_{compost}$+$100_{effluent}$) treatment. With each addition of compost contribution, N_{uptake} declined significantly such that mean N_{uptake} was in the order ($0_{compost}$+$100_{effluent}$) > ($25_{compost}$+$75_{effluent}$) > ($50_{compost}$+$50_{effluent}$) > ($25_{compost}$+$75_{effluent}$) > ($100_{compost}$+$0_{effluent}$) with the mean N_{uptake} of 142, 132, 111, 97 and 86 kg ha^{-1} respectively ($p < 0.05$). This observation was consistent with the results of dry matter yield reported in **Section 4.3.2.1**.

N application rates also influenced N_{uptake}. N_{uptake} was as expected significantly higher ($p < 0.05$) at the N application rate of 150 kg N ha^{-1}. The mean N_{uptake} for N application rate of 75 and 150 kg total N ha^{-1} were 101 and 127 kg N ha^{-1}. N availability was therefore enhanced at an application rate of 150 kg total N ha^{-1}. Comparing N_{uptake} between the two years of the experiment showed that from 2010/11 to 2011/12, N_{uptake} declined from 128 to 75 kg N ha^{-1} when N application rate was 75 kg N ha^{-1}. At N application rate of 150 kg N ha^{-1}, N_{uptake} declined from 146 to 108 kg N ha^{-1}. This interaction of time and N application rate was significantly different ($p = 0.00$).

The four-way interaction of soil types, N application rates and combinations of compost and STSE with time significantly influenced N_{uptake} ($p < 0.05$) **(Figure 4-10 and Figure 4-11**). However, analysis of ryegrass cuts made in 2010/11 (first year) showed that N_{uptake} was not significantly influenced by the combinations of compost and STSE. This implied that at this particular moment, the levels of readily available N in all combinations of compost and STSE was similar. In treatments with compost alone (($100_{compost}$+$0_{effluent}$)), the initially higher NH_4^+-N concentration in greenwaste compost reported in **Chapter 3** provided enough NO_3^--N for plant growth. 1879 kg Mineral N ha^{-1} was provided through compost in the treatment (($100_{compost}+0_{effluent}$)) at the start of the experiment. Above all, pre-treatment of soil (drying, sieving and rewetting) can also promote initial N mineralisation (Cordovil et al., 2005) which plants can take advantage off immediately after application of the amendments.

4.3.2.4 Nitrogen use efficiency

Nitrogen use efficiency (NUE) was estimated using Partial Factor Productivity (PFP_e). PFP_e of applied N is a measure of N productivity. It is also an indicator of potential availability and loss of N within the soil. As described in **Chapter 2** (**Literature review**), PFP_e estimates NUE by considering total output of plant DM and total input of N applied. PFP_e is an aggregate efficiency index that includes contribution to yield derived from uptake of indigenous soil N (Zhu et al., 2011). The results of PFP_e analysis have been presented in **Table 4-5**. PFP_e was determined using **Equation 4-1.**

$$PFP_e = \frac{Y_N}{F_N} \qquad \textbf{Equation 4-1}$$

Where Y_N and F_N are for crop yields and amount of N applied in kg ha^{-1} respectively.

Analysis of PFP_e for 2010/11 and 2011/12 showed that PFP_e was significantly influenced by the soil type, N application rates and the combinations of compost and STSE. The mean PFP_e in sandy loam (47 kg DM kg^{-1} applied N) was significantly lower ($p < 0.05$) as compared to clay loam soil (75 kg DM kg^{-1} applied N). Comparing N application rates, PFP_e decreased with increasing N application rates. The mean PFP_e across the soil types and the combinations of compost and treated effluent was 76 and 46 kg DM kg^{-1} applied N for N application rates of 75 and 150 kg total N ha^{-1} respectively.

Table 4-5 Nitrogen use efficiency expressed as Partial Factor Productivity (kg DM kg^{-1} applied N) for combinations of compost and STSE in the sandy loam and the clay loam soils for 2010/11 and 2011/12 (p = 0.35).

Soil type	Combinations of compost and STSE (%)	2010/11	2011/12	2010/11	2011/12
		75 kg N ha^{-1}		150 kg N ha^{-1}	
Sandy loam	$0_{compost}+100_{effluent}$	72^{a}	73^{a}	49^{a}	54^{a}
	$25_{compost}+75_{effluent}$	61^{b}	58^{b}	40^{b}	48^{b}
	$50_{compost}+50_{effluent}$	53^{c}	58^{cb}	31^{c}	37^{c}
	$75_{compost}+25_{effluent}$	50^{dc}	47^{d}	31^{cd}	30^{d}
	$100_{compost}+0_{effluent}$	54^{ec}	42^{ed}	26^{e}	20^{e}
Clay loam	$0_{compost}+100_{effluent}$	126^{a}	81^{a}	76^{a}	53^{a}
	$25_{compost}+75_{effluent}$	127^{a}	76^{ab}	67^{b}	54^{a}
	$50_{compost}+50_{effluent}$	122^{a}	73^{b}	63^{cb}	46^{b}
	$75_{compost}+25_{effluent}$	115^{b}	62^{c}	65^{db}	34^{c}
	$100_{compost}+0_{effluent}$	113^{cb}	52^{d}	57^{e}	28^{d}

PFP_e values followed by different letters in a column are significantly different ($p < 0.05$).

As presented in **Section 4.3.2.2 and 4.3.2.3**, N_{upake}, DM yield and TN_{plant} were significantly higher at N application rate of 150 kg total N ha^{-1} as compared to 75 kg total N ha^{-1}. However, despite higher N_{upake}, DM and TN_{plant}, N productivity as evidenced by the PFP_e was low at N application rate of 150 kg total N ha^{-1}. At this point, regardless of the experimental conditions in the glasshouse and only considering PFP_e, the application rates were not optimal N application rates. N source played a crucial role in influencing NUE. Since compost N is mostly in organic form with its net N mineralisation significantly low (Hadas and Portnoy, 1994a), it is possible that not all the total N applied through compost was made available to the ryegrass. This explains why mean PFP_e reduced significantly with increasing compost contribution in both soils **Figure 4-12** and **Figure 4-13**.

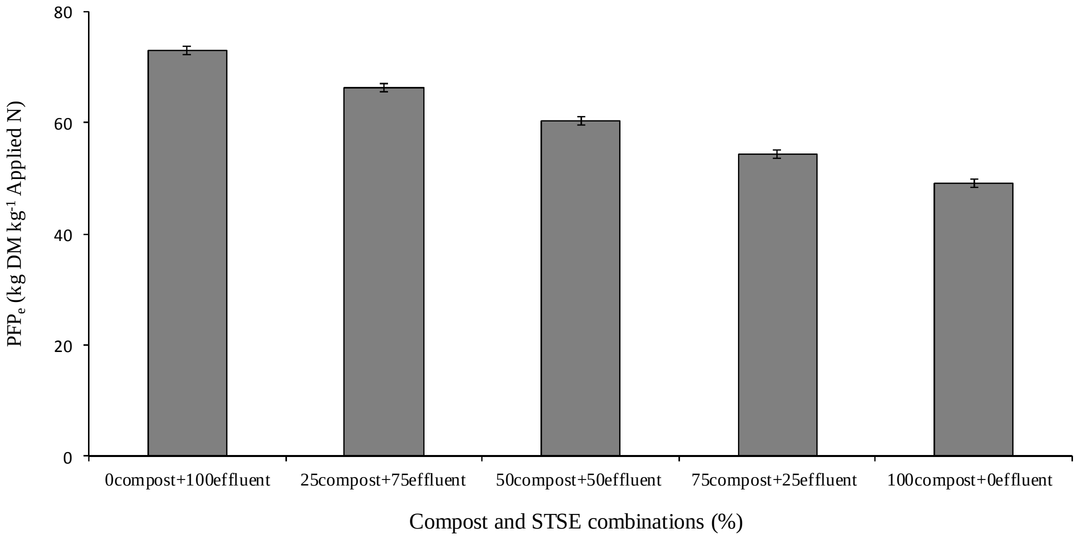


Figure 4-12 Effect of the combinations of compost and STSE on PFP_e. The values shown in the graph correspond to the mean value for the two nitrogen application rates and soil types used ($p = 0.00$). Error bars represent ± SEM.

Using the PFP_e approach, NUE declined significantly with increasing contribution of compost in combinations of compost and STSE (**Figure 4-12**). The lowest mean PFP_e (49 kg DM kg^{-1} applied N) was recorded in the treatment ($100_{compost}$+$0_{effluent}$) while the treatment ($0_{compost}$+$100_{effluent}$) had the highest mean PFP_e of 73 kg DM kg^{-1} applied N. The effect of the interaction of compost and effluent combinations and soil type on PFP_e was significant (**Figure 4-13**). However in both soils, the treatment ($100_{compost}$+$0_{effluent}$) registered the lowest mean PFP_e of 36 and 63 kg DM kg^{-1} applied N in the sandy loam and clay loam soils respectively.

Repeated measures ANOVA between the years (2010/11 and 2011/12), showed a significant decline of PFP_e. Across all treatments and soil types, PFP_e declined significantly in the second year by 27%. This observation was consistent with the results of dry matter yield. On average, the decline was from treatments with a higher contribution of compost than effluent (($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$)). The mean PFP_e decline was 19, 20, 20, 34 and 43% for ($100_{compost}$+$0_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$), ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively. Apart from the low net N mineralisation rates reported in **Chapter 3**, poor compost incorporation at the start of the second year left compost exposed on the surface affecting microbial provision of substrate. The soil tillage done prior to compost amendment to facilitate compost application at the start of the second year was restrictive as the pots still had ryegrass growing. The changes in the micro-climate inside the glasshouse also affected availability of N and growth of ryegrass.

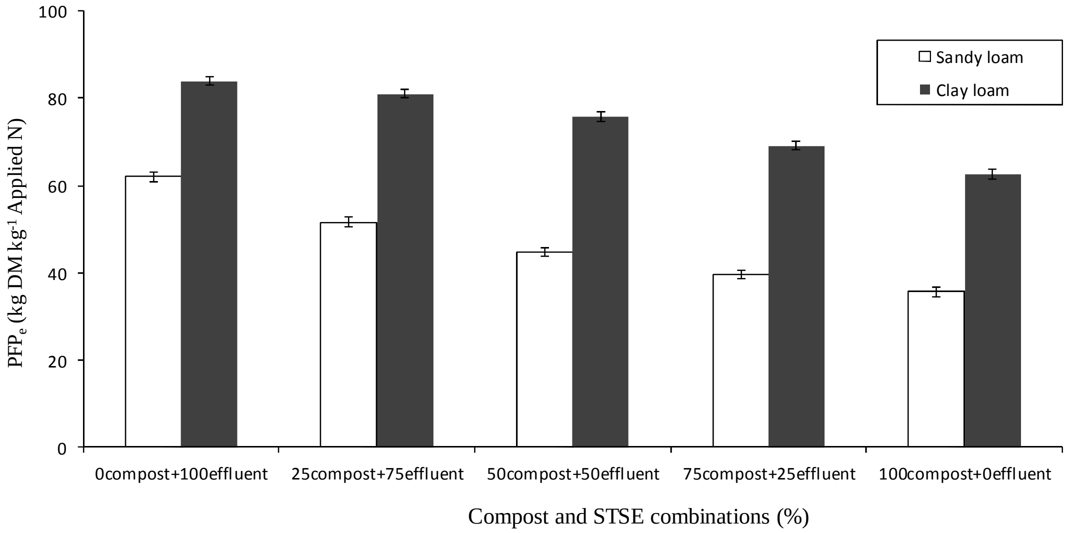


Figure 4-13 Effect of the interaction of combinations of compost and STSE and soil type on PFP_e (p = 0.00). The values shown in the graph correspond to the mean value for the two nitrogen application rates and soil types used (p = 0.00). Error bars represent ± SEM.

4.3.3 Soil properties

4.3.3.1 Soil mineral nitrogen

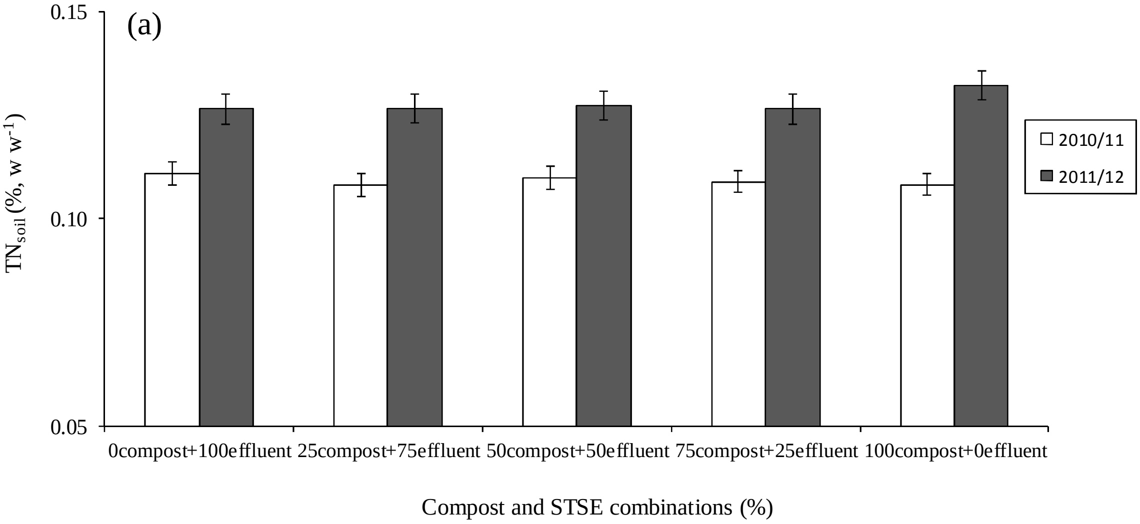
Soil mineral nitrogen (SMN) was regarded as the sum of NO_3^--N and NH_4^+-N. Initial analysis of the soils before the study showed that the mean SMN in the clay loam was 68.8 mg kg^{-1} while in sandy loam it was 8.5 mg kg^{-1}. However, analysis of SMN within the two years of the experiment showed small quantities of mineral N in the soils. SMN was not significantly influenced by the N application rates and the combinations of compost and STSE. Significant differences were observed between the soil types with sandy loam having higher SMN of 1.32 mg kg^{-1} and clay loam, 0.95 mg kg^{-1}. Overall, SMN reduced with time ($p < 0.05$). Mean SMN (for the combinations of compost and STSE, soil types and N application rates) reduced from 1.52 to 0.75 mg kg^{-1}. However, despite the significant differences, the quantities recorded were so low such that when expressed per hectare, the SMN in sandy loam and clay loam was 2.77 and 1.99 kg ha^{-1}. Similar observations were made in comparable studies by Kokorra (2008) and Antille (2011).

The lower SMN observed in all treatments signified that the last ryegrass cuts in both years were made at a time when the grass was actively growing. In actively growing grass, any mineralised N is utilised by plants for growth hence low SMN. The threat of residual N leaching is significantly reduced when SMN is too low in the soil.

4.3.3.2 Soil total nitrogen

Statistical analysis of soil total nitrogen (TN_{soil}) indicated that there were significant differences between soil types. The mean TN_{soil} was significantly ($p < 0.05$) higher in the clay loam when averaged across all the treatments. In the clay loam, the mean TN_{soil} was 0.185% w w^{-1} while in sandy loam it was 0.119% w w^{-1}. Overall, TN_{soil} was not significantly influenced by the combinations of compost and STSE and the N application rates ($p > 0.05$). With time, there were significant differences in TN_{soil} in both soils. Mean TN_{soil} increased significantly from 0.109 to 0.128% w w^{-1} in sandy loam and from 0.174 to 0.196% w w^{-1} in the clay loam from the first year to the second year. With respect to initial TN_{soil} before the experiment, TN_{soil} increased by 6% and 40% in the sandy loam and clay loam soils respectively.

TN_{soil} was also influenced by the interaction of time, soil type and the combinations of compost and STSE ($p = 0.03$). The results of the interaction of time, soil type and the combinations of compost and STSE have been presented in **Figure 4-14**. Similarly, the interaction of time, soil type and the combinations of compost and STSE was significantly different ($p = 0.01$). As presented in **Section 4.3.2.1**, average DM yield was significantly higher in the clay loam which also happened to have significantly higher TN_{soil} than in sandy loam soil. In this case, TN_{soil} offers a likely explanation to the higher DM in the clay loam soil. However the C/N ratio was significantly lower in the sandy loam as compared with the clay loam soil ($p = 0.001$). In the sandy loam, C/N ratio was 11.12 as compared with 11.29 for the clay loam. Springob and Kirchmann, (2003) reported that a C/N ratio of 15 is a critical limit separating soil groups with higher or lower N release. Though the C/N ratio of the two soils was significantly different, the magnitude of the difference was too small to have any meaningful effect in practice. With respect to initial C/N of the soils before the experiment, C/N increased by 3% in the sandy loam and decreased by 5% in the clay loam.



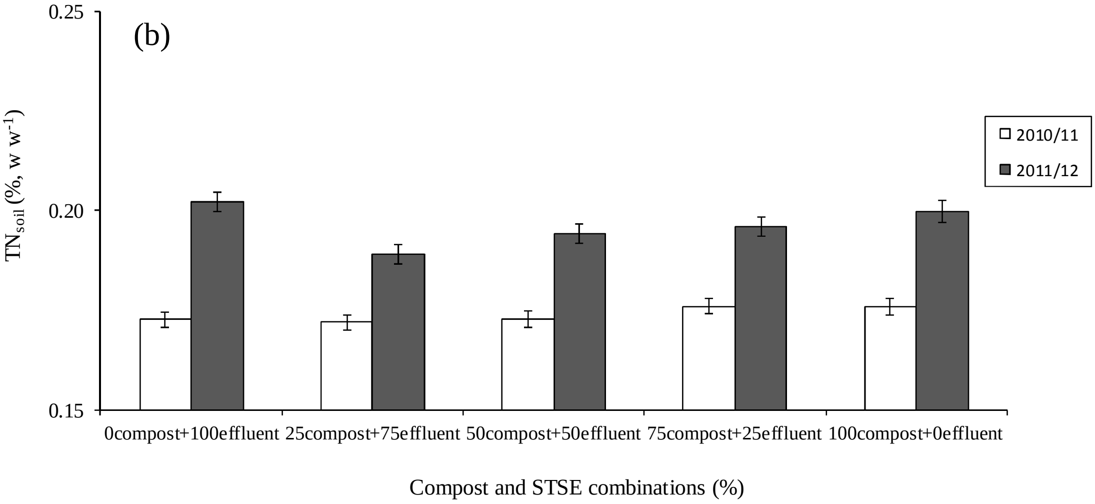


Figure 4-14 Influence of the interaction of the combinations of compost and STSE, soil type and time on TN_{soil} in a) sandy loam and b) clay loam. The values shown in the graphs correspond to the mean value for the two nitrogen application rates used (p = 0.03). Error bars represent ± SEM.

4.3.3.3 Soil total carbon

Total soil carbon was significantly influenced by the soil types and N application rates. Soil C was significantly higher in the clay loam at 2.08% as compared to 1.31% in the sandy loam (p = 0.00). Increasing N application rates from 75 to 150 kg total N ha^{-1}, increased soil C from 1.68 to 1.72% ($p < 0.05$). Carbon availability is an important factor controlling N cycling in soil. According to Hart et al., (1994) microbial demand for N declines as C availability declines. However, compared to the initial native soil C in the sandy loam (1.29%) and clay loam (1.68%) soils, soil C increased in all treatments by the end of the experiment. In the sandy loam, mean soil carbon was 1.36, 1.37, 1.36, 1.36 and 1.41% for the ($0_{compost}+100_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) respectively. Mean soil C in the sandy loam was not significantly different for all the combinations of compost and STSE. In the clay loam, soil carbon was mean 2.14, 2.05, 2.08, 2.19 and 2.13% for the treatments ($0_{compost}+100_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) respectively. Mean soil C in the clay loam for the treatments ($0_{compost}+100_{effluent}$) and ($75_{compost}+25_{effluent}$) were significantly higher than all the other combinations of compost and STSE.

4.3.3.4 Soil extractable phosphorous and total phosphorous

Soil extractable P was analysed from soil samples at the end of the first year (2011) and end of the second year (2012). The combinations of compost and STSE did not significantly influence soil extractable P in both soils ($p > 0.05$). Soil extractable P was however significantly influenced by the soil types ($p = 0.00$) and N application rates ($p = <0.01$). Soil extractable P increased significantly with time ($p = 0.00$). Mean soil extractable P at the end of the first year and second year were 23.4 and 31.2 mg kg^{-1} respectively for the combinations of compost and STSE, N application rates and soil types.

Mean soil extractable P was 34.4 mg kg^{-1} in the sandy loam while in the clay loam soil, mean soil extractable P was 20.1 mg kg^{-1} for the various combinations of compost and STSE and the N application rates. When compared to the background levels of extractable P in the soils before the study (**Chapter 3**), soil extractable P reduced by 15% and 7% in the sandy loam and the clay loam respectively.

With respect to N application rates, soil extractable P increased when N application rate increased from 75 to 150 kg N ha^{-1}. Soil extractable P increased significantly from 26.7 to 27.9 mg kg^{-1} when N application rate was increased from 75 to 150 kg N ha^{-1}. **Figure 4-15** shows the influence of the interaction of soil type and combinations of compost and STSE on soil extractable P. Non-significant influence was observed for this interaction ($p = 0.13$).

Similarly, total soil P was significantly influenced by the soil type. In the sandy loam, mean total P was 0.95 g kg^{-1} while in the clay loam it was 0.62 g kg^{-1} for the various combinations of compost and treated effluent and the N application rates. Availability of soil phosphorous amongst others is governed by the quantity of clay minerals available in the soil. Soils containing large quantities of clay have a large surface area exposed to retain phosphorus (Tisdale et al., 1990). As reported in **Chapter 3**, clay content in the sandy loam and clay loam soils used in this study were 12% and 29% respectively while cation exchange capacity (CEC) in the sandy loam and clay loam were 10 and 17 cmol+ kg^{-1} respectively. With time, total P increased by 15% from the first year to the second year for the various combinations of compost and treated effluent, N application rates and soil types. The amendments (compost and effluent) affected the levels of total P in the soil during the experimental period. But levels of total P were not significantly influenced by the combinations of compost and STSE.

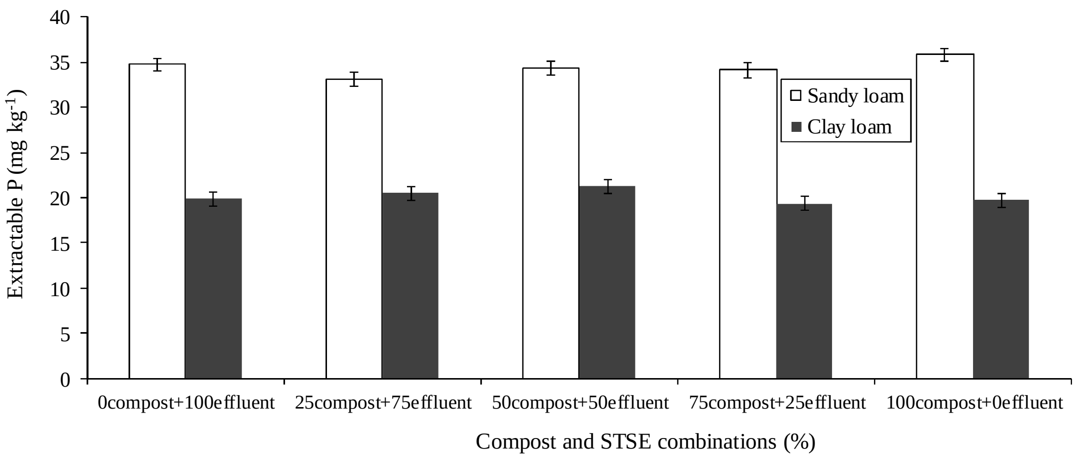


Figure 4-15 Influence of the interaction of combinations of compost and STSE and soil type on soil extractable P. The values shown in the graph correspond to the mean value for the two nitrogen application rates used ($p = 0.13$). Error bars represent ± SEM.

The contribution of final effluent P to soil P was through the soluble P pool. The soluble P pool is important because it is the pool from which plants take up P and is the only pool that has any measurable mobility. A growing crop would quickly deplete the P in the soluble P pool if the pool was not being continuously replenished. Effluent P was likely used up by the growing ryegrass.

4.3.3.5 Soil heavy metals

i. Copper

Statistically analysis of copper (Cu) indicated that there were significant differences between soil types. The mean Cu was significantly ($p < 0.05$) higher in the clay loam when averaged across all the treatments. In the clay loam, the mean Cu was 18.2 mg kg^{-1} while in the sandy loam it was 16.4 mg kg^{-1}. Overall, Cu was not significantly influenced by the combinations of compost and STSE and the N application rates ($p > 0.05$). With time, there were significant differences of Cu. Cu increased significantly from 15.3 to 19.3 mg kg^{-1} from the start (2010) to the end of the pot study in 2012 across both soil types and all combinations of compost and STSE.

ii. Lead

Analysis of lead (Pb) showed that there were significant differences between soil types. The mean Pb concentration was significantly higher ($p < 0.05$) in the clay loam when averaged across the combinations of compost and STSE and N application rates. In the clay loam, the mean Pb was 116.1 mg kg^{-1} while in the sandy loam it was 82.4 mg kg^{-1}. Overall, Pb was not influenced by the combinations of compost and STSE and the N application rates ($p > 0.05$). With time, there were significant differences of Pb. Pb increased significantly from 89 to 110 mg kg^{-1} from the start to the end of the pot study across both soil types and all combinations of compost and STSE.

iii. Chromium

Soil samples were analysed at the start of the study (2010) and at the end of the study in 2012. Analysis of chromium (Cr) indicated that it was significantly influenced by soil type, time and the interaction of application rate and time. Averaged across the N application rates and the combinations of compost and STSE, the mean Cr was significantly higher ($p < 0.05$) in the sandy loam. In the sandy loam, the mean Cr was 37.1 mg kg^{-1} while in the clay loam it was 31.8 mg kg^{-1}. Cr increased significantly from 29.2 to 38.2 mg kg^{-1} at N application rate of 75 kg ha^{-1} while at N application rate of 150 kg ha^{-1}, Cr increased from 26.7 to 43.6 mg kg^{-1} by the end of the pot experiment. Compared to the start of the experiment in 2010, Cr increased by 46% in 2012 (mean for the combinations of compost and STSE, soil types and N application rates). Overall, Cr was not significantly influenced by the combinations of compost and STSE and the N application rates ($p > 0.05$).

iv. Nickel

Statistically analysis of nickel (Ni) indicated that there were non-significant differences due to the main treatment factors (soil type, N application rates and the combinations of compost and STSE). Ni was significantly influenced by the interaction of experimental time and N application rate. The mean Ni averaged across the combinations of compost and treated effluent and soil type increased significantly at N application rate of 75 kg ha^{-1} from 12.8 to 16.7 mg kg^{-1} at the end of the study. Non-significant differences were observed at N application rate of 150 kg ha^{-1}.

v. Zinc

The effects of combined application of compost and STSE and N application rates on zinc (Zn) were not significantly different ($p > 0.05$). Zn was significantly influenced by the soil type. It was significantly higher in the clay loam (77 mg kg^{-1}) as compared to the sandy loam soil (62 mg kg^{-1}).

4.3.3.6 Soil pH

Soil pH was analysed from soil samples collected after each ryegrass cut in the first year (2010/11) and at the end of the second year, 2012. The results presented in this section are for the analysis of soil pH for the end of first year and second year (2012).

Initial analysis of soil pH before the study indicated that the two soil types had significantly different soil pH ($p < 0.05$). Soil pH was significantly higher in the clay loam (7.6) than in the sandy loam (6.8). At the end of the experiment, soil pH was significantly influenced by soil types. In the sandy loam and clay loam, mean soil pH was 7.9 and 8.2 respectively, showing an increase when compared to the initial soil pH of the respective soils.

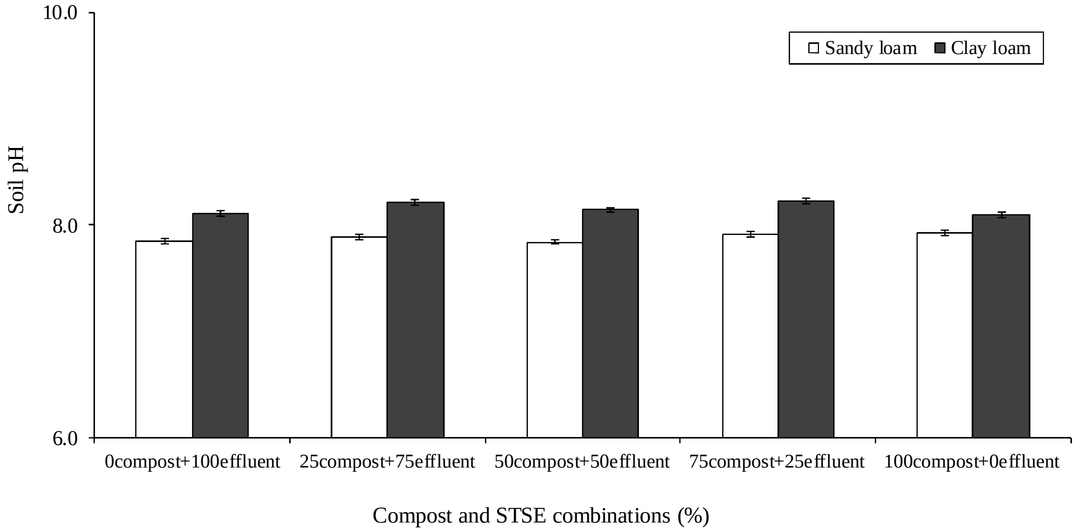


Figure 4-16 Influence of the interaction of compost and STSE combinations and soil type on soil pH. The values shown in the graph correspond to the mean value for the two nitrogen application rates used (p = 0.12). Error bars represent ± SEM

Soil pH was also influenced by the combinations of compost and STSE ($p < 0.05$). Soil pH was significantly higher in the treatments ($25_{compost}+75_{effluent}$) and ($75_{compost}+25_{effluent}$) than in the treatments ($0_{compost}+100_{effluent}$), ($50_{compost}+50_{effluent}$) and ($100_{compost}+0_{effluent}$). However, though the levels of soil pH for the combinations of compost and STSE were statistically different, the differences were very marginal. **Figure 4-16** shows the non-significant influence of the interaction of combinations of compost and STSE and soil types on soil pH (p = 0.12).

4.3.3.7 Soil organic matter

Soil samples were analysed for organic matter (OM) at the start of the study (2010), end of the first year (2011) and the end of the study (2012). The results presented in this section are for the soil analyses at the start and end of the study.

Soil organic matter content was significantly influenced by soil types and the interaction of the combination of compost and STSE and N application rates. Mean soil OM was significantly higher (p = 0.00) in the clay loam soil (4.70%) as compared to sandy loam (3.38%). As shown in **Figure 4-17**, the interaction of soil types and compost and STSE combinations were not significant (p = 0.37).

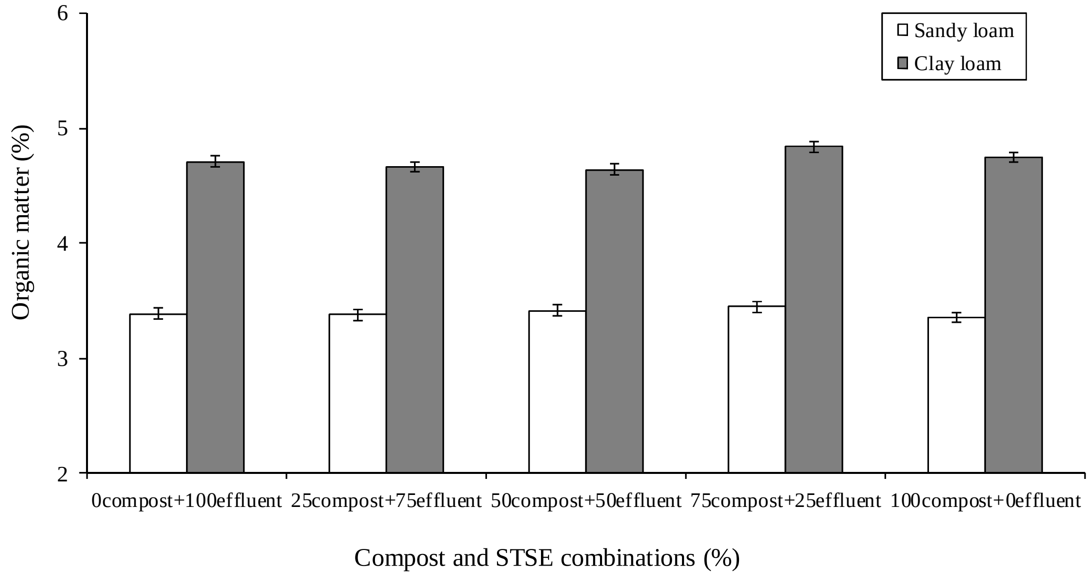


Figure 4-17 Influence of the interaction of combinations of compost and STSE and soil type on soil organic matter. The values shown in the graph correspond to the mean value for the two nitrogen application rates used (p = 0.37). Error bars represent ± SEM

Soil OM increased with time irrespective of the combinations of compost and STSE, soil type and N application rates. As compared to the start of the study, soil OM increased by 9% and 4% in the sandy loam and clay loam soil at the end of the study in 2012. But there were non-significant differences on soil OM due to the combinations of compost and STSE (p = 0.08).

4.3.3.8 Soil microbial biomass

Soil microbial biomass nitrogen (MBN) and C (MBC) were analysed in the sandy loam and clay loam soil samples before the study and at the end of the study in 2012. The results reflect microbial biomass in the soil with respect to N and C at the end of the 2-year study period. At the end of the study, MBN was significantly influenced by the combinations of compost and STSE (p = 0.03).

The mean MBN for the treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) were 18 and 17 mg kg^{-1} respectively (**Figure 4-18**). These treatments had a significantly higher MBN than the treatments ($0_{compost}$+$100_{effluent}$) and ($100_{compost}$+$0_{effluent}$). The proportion of compost in a combination of compost and STSE played a significant role in influencing

MBN. Increasing the contribution of compost reduced MBN. Despite the fact that microbial biomass is strongly related to organic matter content of the soil (Zhang et al., 2005), MBN was not significantly influenced by the soil type despite as reported in **Section 4.3.2.7** that soil OM was significantly higher in the clay loam as compared with the sandy loam.

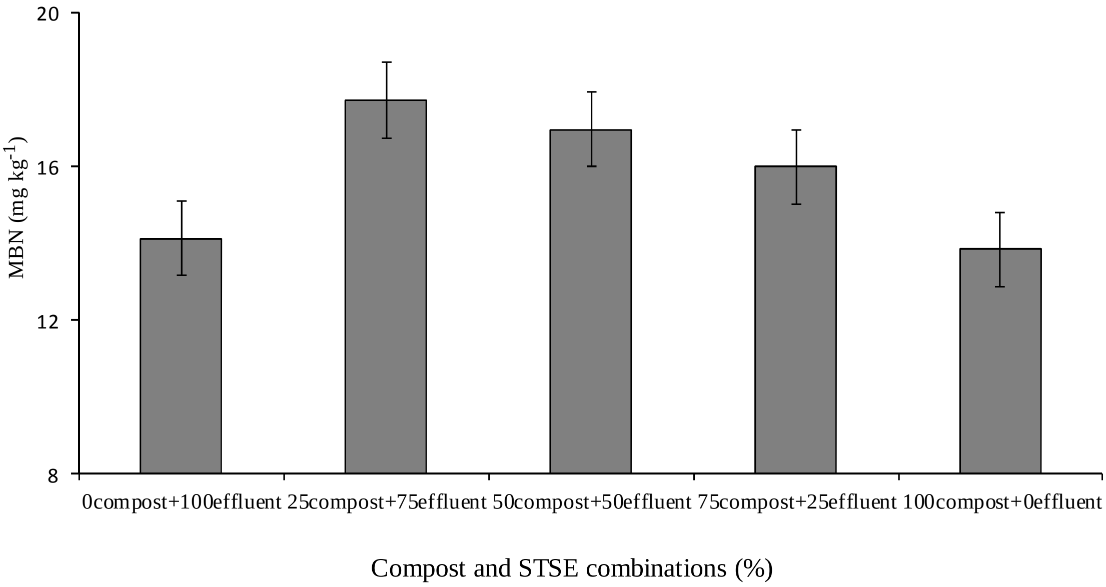


Figure 4-18 Influence of the interaction of combinations of compost and STSE on MBN. The values shown in the graph correspond to the mean value for the two nitrogen application rates and soil types ($p = 0.03$). Error bars represent ± SEM

Landgraf et al., (2002) stated that systems with higher organic matter inputs and easily mineralisable organic carbon tend to have higher microbial biomass contents because they are the preferred energy sources for microorganisms. Statistical analysis showed that MBC was not significantly influenced by N application rates ($p = 0.99$) and soil type ($p = 0.63$) but rather by combinations of compost and STSE ($p < 0.05$). Increasing the contribution of compost in a combination of compost and STSE resulted in an increase of MBC (**Figure 4-19**). MBC was significantly higher in the treatment with compost alone, ($100_{compost}+0_{effluent}$). From the initial analysis of the greenwaste compost in **Chapter 3**, carbon content in the compost was *c*. 22% while organic matter was 38%. Treatments with higher contribution of compost had higher MBC as the microbes were supplied with enough substrates as energy source for microbial activities but the lower input of N from these treatments limited availability of N in the soil. On the other hand, in treatments with effluent alone, microbes accessed readily available N from STSE but had limited substrates due to absence of organic amendment (compost) which had enough carbon and organic matter.

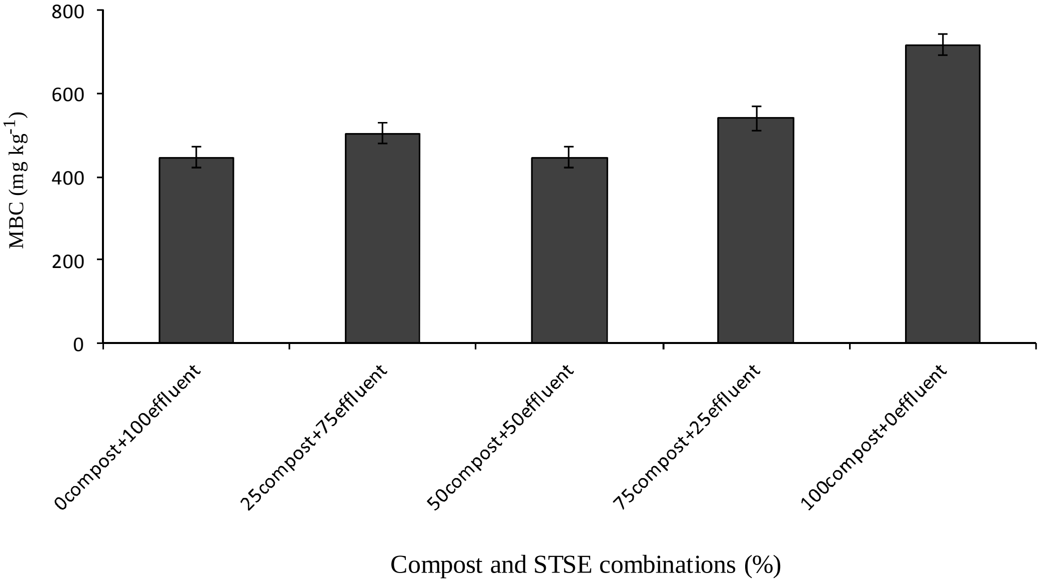


Figure 4-19 Influence of the interaction of combinations of compost and STSE on MBC. The values shown in the graph correspond to the mean value for the two nitrogen application rates and soil types ($p = 0.03$). Error bars represent ± SEM.

4.4 Overall discussion

The pot experiment was effective to measure, assess and explore the effects of irrigating STSE on soils amended with greenwaste compost on ryegrass production and soil physical properties. The response to the integration of compost and STSE on ryegrass production and soil physical and chemical properties within the two years of the study was influenced by the soil types and the N application rates.

Ryegrass DM yields varied annually between the two years and also varied between the ryegrass cuts made in the two years. Ryegrass DM in this study referred to plant material cut 2 cm above the surface of the soil. Increasing N application rates from 75 to 150 kg total N ha^{-1} significantly increased mean DM from 5685 to 6808 kg DM ha^{-1} across the soil types and combinations of compost and STSE. Ryegrass DM increased by 22% after increasing N application rate to 150 kg total N ha^{-1}. This increase in DM yield was low considering that N application rate increased by 100%. Grass crops respond linearly to N application rates within the range of 0 to 300 kg ha^{-1} (Antille, 2011; Morrison et al., 1980). Increased ryegrass DM yield can be expected to increase with the proportion of available soil N that the plants recover in the herbage (Schenk, 1996). According to Wilkins et al., (2000) this is highly desirable as it could help to reduce immediate losses of N to the environment.

Assessment of NUE between the N application rates showed significant differences. Comparing the N application rates, NUE (measured as PFP_e) decreased with increasing N application rates. The mean PFP_e across the soil types and the combinations of compost and treated effluent were 76 and 46 kg DM kg^{-1} applied N for 75 and 150 kg total N ha^{-1} respectively. Relating PFP_e to DM yield showed that the overall DM yield (for the compost-effluent combinations and soil types) for N application rate of 75 kg total N ha^{-1} was low despite a significantly higher PFP_e. A higher PFP_e can sometimes result in unacceptably low DM yield production (Zhu et al., 2011). Actually, despite a higher PFP_e at N application rate of 75 kg total N ha^{-1}, TN_{plant} was less than 1.8% which is the critical concentration of N in plant herbage to achieve 90% of maximum ryegrass yields (Robinson, 1996) in (Evers, 2002). In this study, the higher N application rate was based on the recommendations made by MAFF (2000) for soils with low soil N supply for a single cut. While the low N application rate of 75 kg total N ha^{-1} was aimed at exploring the effect of the combinations of compost and STSE at lower than optimal N requirement for grass.

The response of ryegrass DM yield to the different soil types was significantly different. Higher DM yield was observed for combinations of compost and STSE in clay loam. The mean total ryegrass DM yield was 15,222 kg DM ha^{-1} in the clay loam while in the sandy loam it was 9764 kg DM ha^{-1}. The higher DM yield in the clay loam may have been an indication that the integration of compost and STSE worked well in the clay loam soil but considering that initial soil characteristics reported in **Chapter 3** showed that the clay loam soil was more fertile than sandy loam; a definite conclusion cannot be drawn.

Wilkins et al., (2000) indicated that for productive grazing animals the minimum level of N in herbage required is 20 g N kg^{-1} while for higher producing dairy cows, the range required for N herbage is 2.2 – 2.7% (Aavola and Karner, 2008). For dairy cows grazing on low N grass, protein supply may limit milk production (Delaby et al., 1996). TN_{plant} in the clay loam (in relation to required N herbage for animal feed) for the integration of compost and STSE at both 75 and 150 kg N ha^{-1} in the first year (2010/11) were above the minimum requirement for both productive grazing and dairy cattle. TN_{plant} was *c*.24, *c*.22, *c*.23, *c*.21 and *c*.20 g N kg^{-1} DM for ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively at N application rate of 75 kg N ha^{-1}. At N application rate of 150 kg N ha^{-1}, TN_{plant} was *c*.25, *c*.25, *c*.22, *c*.22 and *c*.22 g N kg^{-1} DM for ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively. The concentration of N in plant material was within the range of 1.3 to 3.3% found by Evers (2002) after combining fertiliser and poultry manure.

In **chapter 3**, N mineralisation patterns for the two soils as a result of the combinations of compost and STSE in the clay loam soil, showed that the release of N from the nutrient sources peaked at 30 days after which N mineralisation was constant. Pre-treatment of soil through drying and rewetting contributes to initial N mineralisation flush (Cordovil et al., 2005). Soil mineral N (NO_3^--N and NH_4^+-N) was therefore readily available during this time. TN_{plant} in 2010/11 decreased with each cut that was made. Mean TN_{plant} for the first cut made in 2010/11 in the clay loam was significantly higher (3.94%) than the second cut (1.87%) and third cut (0.96%) made in the same year. The delay shown by ryegrass plants after cutting in regaining their former rate of tillering can affect not only TN_{plant} but also DM yield (Anslow and Green, 1967). In the sandy loam, TN_{plant} was significantly lower for all the combinations of compost and STSE. This is in agreement with the trend of potential NM_{net} discussed in **Chapter 3**.

Irrigating with STSE alone without compost-N contribution (($0_{compost}$+$100_{effluent}$)) resulted in an increase of ryegrass DM yield in both soils and N application rates. However increasing the amount of compost whilst reducing the amount of STSE irrigated, significantly reduced ryegrass DM yield. The effect of the combinations of compost and STSE on ryegrass DM yield was largely governed by the nutrient source which was in higher proportion. Analysis of total DM yield for the study showed that for the ($0_{compost}+100_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$) and ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) treatments, the mean DM yield (for the two N application rates and soil type) were 15305, 13989, 12166, 11165 and 9840 kg DM ha^{-1}. Provision of readily available N through STSE helped to increase ryegrass DM yield for ($0_{compost}+100_{effluent}$). It was concluded in **Chapter 3** that NM_{net} in the clay loam reduced with increasing quantity of compost in combined application of compost and STSE. The pattern of ryegrass DM yield is therefore in agreement with this conclusion. DM yield reduced with increasing compost proportion in integrated compost and STSE nutrient application.

Build-up of soil OM may be hugely affected if STSE alone is irrigated without any organic amendment e.g. ($0_{compost}+100_{effluent}$). There is a close relationship between the nutrient status of the soil and soil organic matter content. As observed by Goyal (1999), application of compost amendments to soil improves both soil organic carbon and total nitrogen. Soil organic C and N contents provide a measure of soil organic matter status. It has been shown by Chen et al., (2005) that in addition to supplying nutrients from mineralisation of organic matter, the advantages of higher availability of nutrients with soils of higher organic matter contents are multiple.

Statistical analysis of soil OM failed to reflect on the slow build-up of OM in treatments with effluent irrigation without compost amendments (or minimal compost contribution) e.g. ($0_{compost}+100_{effluent}$) and ($25_{compost}+75_{effluent}$). Non-significant differences were found between the various combinations of compost and STSE on soil organic matter. The duration of the study was probably not long enough to establish soil OM trends as influenced by the combination of compost and STSE.

N_{uptake} significantly increased with increasing N application rates in 2010/11 and 2011/12. N_{uptake} was significantly higher in treatments with an application rate of 150 kg N ha^{-1}. In relation to the combination of compost and STSE in the sandy loam, increasing compost contribution in integration of compost and STSE reduced significantly N_{upake} in both years. Similarly in the clay loam, N_{uptake} was significantly influenced by the combinations of compost and STSE. Amount of nutrients intercepted by roots depends on the amounts of available nutrients in the soil volume occupied by roots (Marschner, 1995). Most of the N in STSE is readily available and easily intercepted by plant roots while compost N is organically bound thereby affecting its availability in soil pores. From the fitted linear relationship in **Figure 4-5** and **Figure 4-6**, in 2010/11 N_{uptake} for the ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$) treatments in the clay loam (at 150 kg N ha^{-1}) was *c.* 255 and *c.* 240 N ha^{-1} respectively while in 2011/12, it was *c.* 142 and *c.* 145 kg N ha^{-1}. This trend showed that with time for the clay loam at N application rate of 150 kg N ha^{-1}, combinations of compost and STSE with least contribution of compost can perform similarly to those treatments with STSE alone (($0_{compost}$+$100_{effluent}$)).

N_{upake} is a critical component of integrated soil fertility enhancement involving STSE irrigation. Higher N_{uptake} capacity is a desirable factor for selecting crops in STSE irrigation (da Fonseca et al., 2007). Segarra et al., (1996) emphasised the need for a cropping pattern that not only utilises the disposed effluent but also consumes all the chemical materials supplied with the effluent. Excessive N in the soil can lead to groundwater contamination affecting human health via consumption of water.

Soil analysis showed that at the end of the two years small quantities of soil mineral N were detected in the soils. This was despite the higher initial SMN measured before the study in the clay loam soil of *c.* 69 mg kg^{-1}. SMN was not significantly influenced by N application rate and combinations of compost and STSE. It was significantly influenced by soil type. The significant difference was actually due to the initially higher SMN in the clay loam soil. The mean SMN (for the two N application rates, soil type and the combination of compost and STSE) in 2010/11 and 2011/12 were 1.52 and 0.75 mg kg^{-1} respectively. In soils with actively growing crops, SMN is mostly undetectable or in very small quantities due to N_{upake} (Passoni and Bonn, 2009; Corrêa, 2004). As reported by Correa (2004) nutrient content in harvested materials provides a better estimate of nutrient supply from a given nutrient source. Chadwick et al., (2000) used N_{uptake} to determine N mineralisation in soil.

The combinations of compost and STSE had no significant influence on most soil properties including soil total N, C, soil extractable P and soil P. TN_{soil} and soil C were both significantly influenced by soil type. Overall, the mean TN_{soil} in the clay loam for the two years was significantly higher (0.185% w w^{-1}) than in the sandy loam (0.119%

w w^{-1}). However, when compared to initial soil characteristics, the rate of build-up of TN_{soil} was slow in the sandy loam (from 0.12% to 0.128% w w^{-1}) compared to the clay loam (0.14 to 0.196% w w^{-1}). Similarly soil C was significantly higher in clay loam (2.08% w w^{-1}) as compared with the in sandy loam (1.31% w w^{-1}). Total C increased with time in both soils when compared to the native soil C in the sandy loam and the clay loam soils. In the sandy loam and clay loam soils, total C increased from 1.29 to 1.37% and from 1.68 to 2.12% (w w^{-1}) respectively. Increase of TN_{soil} and total C in studies involving STSE in most cases have been associated with long term experimental periods at higher STSE application rates (Fonseca et al., 2007b). Significant input of N and C through STSE irrigation over time likely stimulated microbial activity thereby enhancing soil organic matter mineralisation (Leal et al., 2010).

The changes in soil pH were due to the influence of the combinations of compost and STSE. Higher soil pH were found in treatments ($25_{compost}+75_{effluent}$) and ($75_{compost}+25_{effluent}$) as compared the treatments ($0_{compost}+100_{effluent}$), ($50_{compost}+50_{effluent}$) and ($100_{compost}+0_{effluent}$). The differences were marginal but statistically significant. However there was no pattern amongst the combinations of STSE and compost to indicate the actual cause of the soil pH difference. But acidity or alkalinity of soil largely depends on the balance between the positively charged basic cations (mostly Ca^{2+}, Mg^{2+}, K^+ and Na^+) and the negatively charged particles of clay and organic matter (Troeh and Thompson, 2005). The basic cations in STSE in this study were not measured but from a study by Sugiura (2009) using STSE from CUSTP, the concentration of Na^+ was 63.2 mg l^{-1}. Organic matter mineralisation results in the formation of organic and inorganic acids that also provide H^+ to the soil.

Assessment of soil extractable P showed that the combination of compost and STSE did not influence soil extractable P. Soil extractable P was affected by soil type and N application rates. The mean soil extractable P in the sandy loam was 34.4 mg kg^{-1} while in the clay loam; it was 20.1 mg kg^{-1}. Soil extractable P declined as compared to the initial soil extractable P in both the clay loam and sandy loam soils. Ryegrass has been described by Brink et al., (2001) to be one of the most effective temperate grasses to remove soil P because it is very productive and has a higher P concentration. Similarly, total soil P was not influenced by the combinations of compost and STSE. Mean total soil P was higher in the sandy loam P as compared to clay loam soil.

Heavy metal analyses focused on the accumulation of Cu, Zn, Cr, Pb and Ni in the soil due to the combined application of compost and STSE. These elements are generally of concern when compost or STSE is applied to provide water or plant nutrients (Smith, 2009; Lottermoser, 2012). Non-significant treatment differences were found in relation to Zn while the other elements were significantly influenced by the soil type. Pb and Cu were significantly higher in the clay loam soil while Cr was significantly higher in the sandy loam soil. The level of metal accumulation in soils is largely influenced by underlying parent materials, soil type and anthropogenic additions (Lottermoser, 2012). Overall, the combinations of compost and STSE did not influence Cu, Zn, Cr, Pb and Ni concentration in the soil. Initial analysis of STSE showed low to non-detectable levels of heavy metals in the STSE. STSE once treated contain less proportion of heavy metals as most of the heavy metals end up in sludge hence its usage for irrigation of crops (Emongor and Ramolemana, 2004). Heavy metals are also predominant in STSE from industrial areas. CUSTP processes sewage from residential areas with minimum threat of heavy metals.

The combinations of compost and STSE did not provoke an increase of heavy metals in the soils. As compared to the critical and maximum allowable heavy metal concentration, all the heavy metals analysed were within the allowable concentration (**Table 4.6**). By the end of the study (2012), mean (for the two soil types and two N application rates) Cu ranged from *c.*18 to *c.* 21 mg kg^{-1}, Pb was from *c.*102 to *c.*116 mg kg^{-1}, Ni ranged from 14.97 to 16.18 mg kg^{-1} while Zn ranged from 65.24 to 68.69 mg kg^{-1}.

The effects of STSE irrigation on soil heavy metal accumulation depend on various factors such as concentration of heavy metals in sewage and the period of sewage application (Rattan et al., 2005; Tabari and Salehi, 2009). Smith et al., (1996) and Tabari and Salehi (2009), concluded that generally 10 to 50 years is needed for soil heavy metal levels to precede the standard levels but the potential threat of heavy metal accumulation should not be ignored mainly for treatment combination with a higher contribution of STSE e.g. ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$).

Table 4-6 Critical and maximum allowable concentration of heavy metals in soil (Source; Cela and Sumner (2002)**).**

Metal	MAC (EU)[a]	CSTC[b]
	……………mg kg^{-1}……………	
Cu	1000	60-125
Ni	150	100
Pb	100	100-400
Cd	20	3-8

[a] Maximum allowable concentration (European Union)
[b] Critical soil total concentration

Microbial biomass analyses were conducted on the clay loam and sandy loam soils to establish background characteristics of the soils and at the end of the study in 2012. MBN values at the end of the study were significantly influenced by the combinations of compost and STSE. MBN was significantly higher for the ($25_{compost}+75_{effluent}$) and ($50_{compost}+50_{effluent}$) treatments as compared with treatments with STSE alone (($0_{compost}+100_{effluent}$)) and compost alone, ($100_{compost}+0_{effluent}$). At the same time, increasing the contribution of compost in combined application of compost and STSE increased MBC. MBC was significantly higher in treatment with compost alone (($100_{compost}+0_{effluent}$)). A balance is therefore required in terms of supplying enough substrate to microbes and providing optimum levels of N to prevent N immobilisation and sustain microbial growth in the soil. Both MBN and MBC were not influenced by the soil types. However, clay minerals adsorb soil organic materials and form envelopes around bacterial cells, which restrict the degradation of organic materials or offers protection against microbivory (Zhang et al., 2005).

Supplying effluent alone, ($0_{compost}+100_{effluent}$) in soils with low organic matter e.g. sandy loam can result in proliferation of microbes due to the STSE leading to N immobilisation. As new cells are formed, N is used to build up microbial protoplasm leading to decreased levels of inorganic N for crops (Tisdale et al., 1990). In **Chapter 3**, it was concluded that in the sandy loam soil, increasing compost contribution resulted in reduced N immobilisation. Compost supplied decomposable carbon to microbes even though the soils had a huge stock of soil carbon. This explains why at the end of the pot experiment, mean MBN was significantly lower for the treatment ($0_{compost}+100_{effluent}$) as compared to ($25_{compost}+70_{effluent}$) and ($50_{compost}+50_{effluent}$). The difference in between these treatments was the gradual but continuous supply of mineralisable organic carbon through compost when compared to the treatment with effluent alone, ($0_{compost}+100_{effluent}$).

It is not surprising that mean MBC was significantly higher for treatment with compost alone, ($100_{compost}+0_{effluent}$). Mean MBC was 446, 503, 446, 504 and 717 mg kg^{-1} for the treatments ($0_{compost}+100_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) respectively across both soil types and N application rates. The shortfall for the treatment with compost alone, ($100_{compost}+0_{effluent}$) was that since it did not receive effluent irrigation, growth of microbes was potentially slow affecting decomposition of organic matter. This was in agreement with low NM_{net} reported in **Chapter 3** for the clay loam soil in the incubation experiment for the ($100_{compost}+0_{effluent}$) treatment. Decomposition of substrates depends on the right balance of both N and C to provide microbes with energy. For STSE analysis conducted in 2011, mean dissolved organic C of 88 mg l^{-1}. Accumulation of soil organic C in the soil can be observed after application of wastewater rich in organic carbon over a long period of time (Jueschke et al., 2008). Similarly, Kayikcioglu (2012) reported that the amount of soil microbial C increased depending on the duration of wastewater application.

4.5 Conclusion

The main conclusions drawn from the pot study are summarised below;

a) Ryegrass DM yield reduced with increasing contribution of N from compost whilst reducing the amount of STSE irrigated. Irrespective of the N application rates and soil type, DM yield was in the order ($0_{compost}+100_{effluent}$) > ($25_{compost}+75_{effluent}$) > ($50_{compost}+50_{effluent}$) > ($75_{compost}+25_{effluent}$) > ($100_{compost}+0_{effluent}$). Ryegrass DM yields also increased with N application rates.

b) Increasing compost proportion in combinations of compost and STSE reduced N_{uptake}. N_{uptake} increased with N application rate and it was higher in the clay loam soil (150 kg N ha^{-1}) as compared to the sandy loam (78 kg N ha^{-1}). Initial analysis of the soil before the study showed that clay loam soil was much more fertile as compared with the sandy loam.

c) Increasing the contribution of compost in integration of compost and STSE reduced TN_{plant}. TN_{plant} decreased in the order ($0_{compost}$+$100_{effluent}$) < ($25_{compost}$+$75_{effluent}$) < ($50_{compost}$+$50_{effluent}$) < ($75_{compost}$+$25_{effluent}$) < ($100_{compost}$+$0_{effluent}$). Using effluent of similar characteristics especially in the clay loam soil will result in ryegrass herbage N concentrations (for the combinations of compost and STSE) above the minimum requirement for N in herbage for productive grazing and dairy cattle.

d) Using the PFP_e approach, NUE declined significantly with increasing contribution of compost in integrated application of compost and STSE. The lowest PFP_e (49 kg DM kg^{-1} applied N) was recorded in the treatment ($100_{compost}$+$0_{effluent}$) while the treatment ($0_{compost}$+$100_{effluent}$) had the highest PFP_e of 73 kg DM kg^{-1} applied N. NUE declined significantly with increasing N application rate from 75 to 150 kg N ha^{-1}.

e) In the short term, the combinations of compost and STSE did not influence soil physical and chemical properties;

- The combinations of compost and STSE did not induce any significant change of TN_{soil}. But TN_{soil} was affected by the soil types.
- SMN was found to be very low in all pots at all sampling events. The combinations of compost and STSE did not have any significant impact on SMN. This was possibly due to the timing of soil sampling in relation to crop growth.
- Soil extractable P was not influenced by the combinations of compost and STSE nutrient but soil extractable P reduced in both soils.

f) Soil amendment through combinations of compost and STSE did not influence heavy metal accumulation. Cu, Pb and Cr were influenced by soil type and increased with time. The combinations of compost and STSE did not provoke any significant increase of heavy metals in the soils.

g) Soil MBN was related to combinations of compost and STSE nutrient combinations. Apart from the treatment ($0_{compost}$+$100_{effluent}$), MBN decreased with increasing contribution of compost. The least MBN was in the treatment with compost alone (($100_{compost}$+$0_{effluent}$)). MBC increased with increasing proportion of compost. A balance is therefore required in terms of supplying enough substrate to microbes and providing optimum levels of N to prevent N immobilisation and sustain microbial growth in the soil.

5 LYSIMETER STUDY

This chapter presents and discusses the lysimeter study that was carried out from April 2011 to July 2012. Lysimeters were set at College Farm in Silsoe, Bedfordshire, UK and perennial ryegrass *(Lolium perenne)* was grown. The lysimeter experiment was conducted to determine the impact of combined application of compost and STSE on leaching of plant nutrients, fate of soil nutrients and heavy metals enrichment in the test soils. It was also aimed at contributing towards identifying the optimum combination (s) of compost and STSE for sustainable crop production. As such the lysimeter experiment study addressed objectives II, III and IV of the research study presented in **Chapter 1** about leaching of plant nutrients, fate of plant nutrients and optimum combinations of compost and STSE. The soils in the lysimeters were amended with greenwaste compost and irrigated with STSE to supply 150 kg N ha^{-1} according to the various combinations of compost and effluent that were developed.

5.1 Introduction

Lysimeters have been developed for use in soil science and have been used for over 300 years to study the relations between soil, water and plants (Grebet and Cuenca, 1991). The use of lysimeters has been extended to other scientific fields as for example to quantitative and qualitative studies of leaching from waste products or contaminated soils in order to evaluate the environmental impact of these materials (Hansen et al., 2000; Besson et al., 2011). Lysimeters provide a good estimate of real life processes but within controlled conditions. They are good proxy to field scale process but can easily be manipulated.

The lysimeter study was aimed at studying the impacts of nutrient integration from compost and STSE on N leaching, nutrient accumulation, soil properties and ryegrass production. This study augmented the results obtained from the pot study in **Chapter 4**.

The specific objectives of the lysimeter experiment are presented below;

i. To evaluate leaching of plant nutrients due to combined application of compost and STSE.

ii. To assess potential accumulation of plant nutrients and heavy metals in the soil as a result of irrigation of STSE on soils amended with greenwaste compost.

iii. To determine the changes of soil properties as a result of combined application of compost and STSE.

5.2 Materials and methods

5.2.1 Description of lysimeter experiment and weather conditions

The lysimeter experiment was conducted at College Farm in Silsoe, Bedfordshire. **Figure 5-1** shows the location of the lysimeter experiment in the College Farm at Silsoe (52°00'34.40" N, 0°25'59.36" W at an elevation of 71m above sea level). In this study, commercially available sandy loam and clay loam soils were used. The soils were of a different batch to those used in **Chapters 3 and 4** but sourced from the same company. The greenwaste compost used was of the same batch as used in the incubation study (**Chapter 3**). Soil texture was verified by analysing the soil samples using the pipette method (Avery and Bascomb, 1982; BSI, 1990).

The soils (sandy loam and clay loam) used were not air dried but were already sieved through a 10 mm mesh screen by the commercial supplier. Using a bulk density of 1400 kg m^{-3}, about 280 kg of soil was packed in the lysimeters on top of a layer of gravel (8 cm) at the bottom of the lysimeter to facilitate drainage. Lysimeters were developed from plastic barrels of 200 L. **Figure 5-2** present a cross sectional diagram of the lysimeters. The layout of the lysimeters has been presented in **Figure C-1** (**Appendix)**.

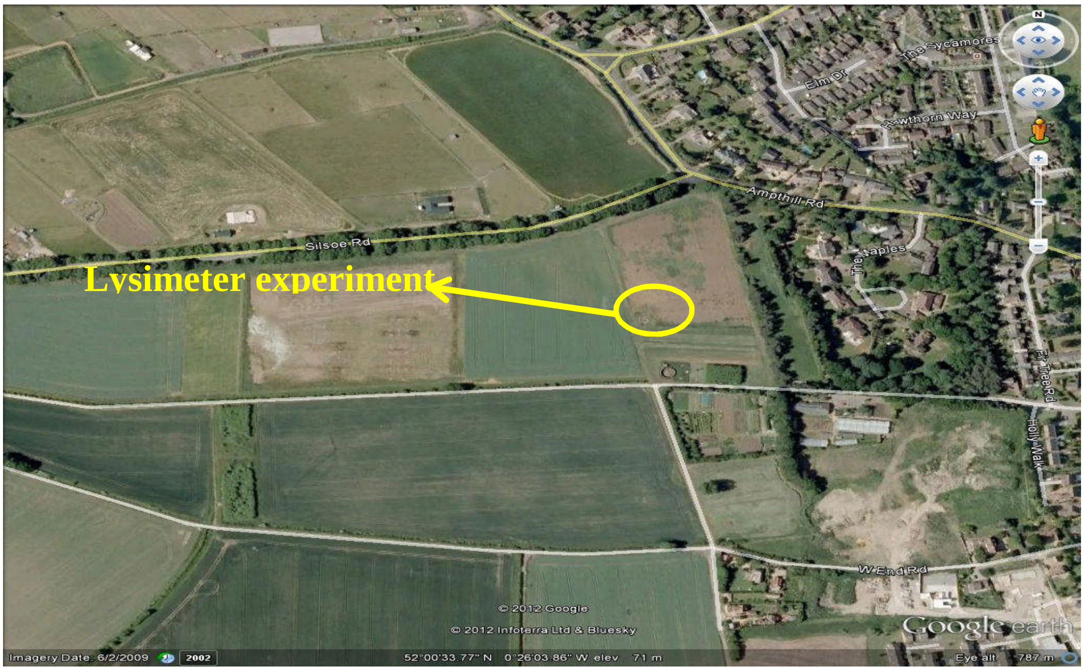


Figure 5-1 Arial view of the location of the lysimeter experiment in College Farm at Silsoe, Bedfordshire, UK (Source: Google Earth).

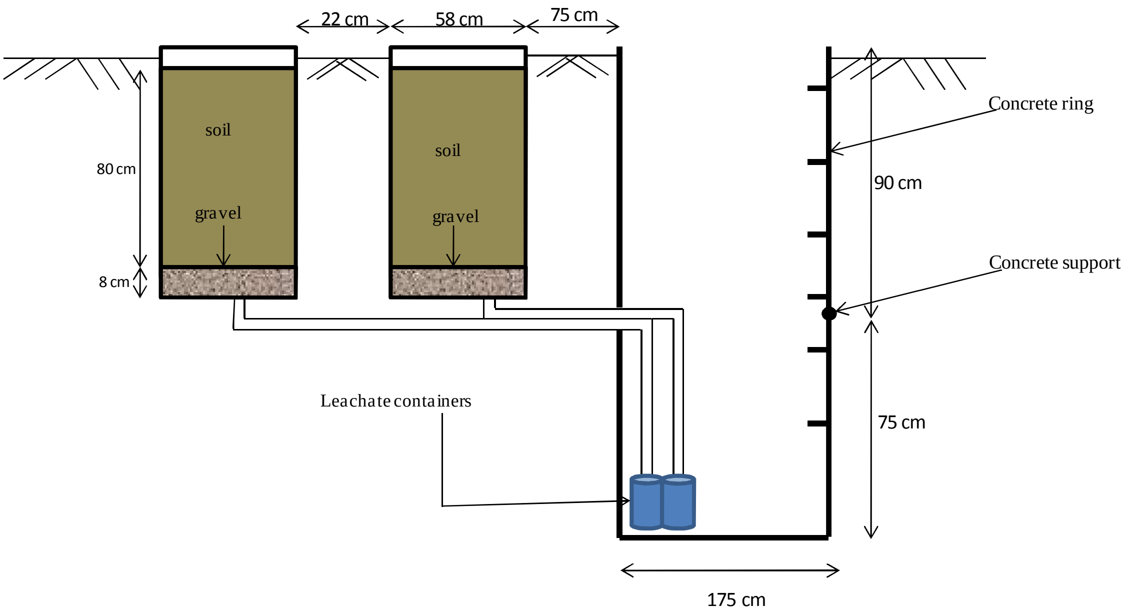


Figure 5-2 Cross sectional view of the lysimeter system at Silsoe showing the design dimensions.

Lysimeters were laid in a circular pattern with leachate collection containers (5 l capacity) located inside the concentric rings that formed the central part of the lysimeter design (**Figure 5-3**). Leachate was collected through plastic drain pipes (20 mm in diameter) fitted at the bottom of each lysimeter. On the inside of the concrete rings, steps were fitted for easy access of the leachate collection containers.

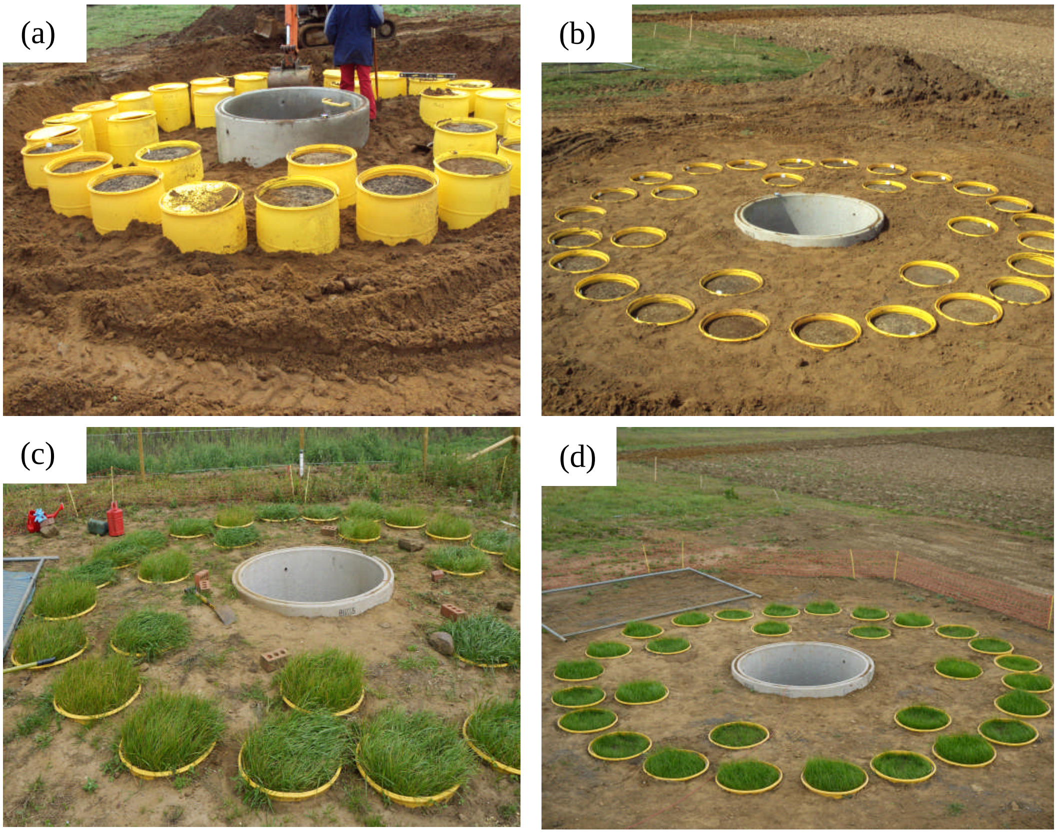


Figure 5-3 Pictorial overview of the lysimeter experiment showing a) establishment of the lysimeter, b) installed lysimeter and c) and d) established ryegrass.

The lysimeter experiment involved the use of triplicate samples in a randomised complete block design of two soil types (sandy loam and clay loam) and five combinations of compost and STSE: (($0_{compost}$ + $100_{effluent}$), ($25_{compost}$ + $75_{effluent}$), ($50_{compost}$ + $50_{effluent}$), ($75_{compost}$ + $25_{effluent}$) and ($100_{compost}$ + $0_{effluent}$)) which resulted in a total of 30 lysimeters. In this study, N application rate of 150 kg N ha^{-1} was adopted for ryegrass. N application rate of 150 kg N ha^{-1} corresponds to the highest N application rate for grass silage in soils with low soil N supply status (MAFF, 2000). A summary of the combinations of compost and STSE developed, projected and actual quantities of compost and STSE applied has been presented in **Section 5.2.3**. The quantity of compost applied was calculated using the surface area of the lysimeter of $0.28m^2$. The quantity of STSE applied depended on total N in the STSE. During preparation of the lysimeters, greenwaste compost was mixed with soil in the top 10 cm soil depth using a commercial cement mixer for thorough mixing. A similar ryegrass seeding rate as reported in **Chapter 4** of 4 g (seeds) m^{-2} was used. Sowing was conducted on 1st April 2011 and germination was recorded approximately 5 days later. Irrigation with STSE started on 24th May 2011. The lysimeter experiment continued up until 31st July 2012. **Table C.1-1** (**Appendix)** summarises the experimental activities (ryegrass cutting, leachate sampling and STSE irrigation) by dates for the duration of the lysimeter experiment.

Determination of the amount of STSE or water to irrigate was based on readings taken from an ETgage™ **(Chapter 3)** and rain gauge installed within the lysimeters experimental yard. More details have been provided in **Section 5.2.3**. Air temperature data was obtained from Clifton Weather Station (Latitude: 52:02:02N and Longitude: 0:17:54W).

5.2.2 Measurement and analysis

5.2.2.1 Soil and compost analyses

The two soil types and greenwaste compost used in the lysimeter experiment were sampled and analysed before the start of the experiment to establish baseline physical and chemical characteristics of the materials. **Table 5-1** provides a summary of the analyses conducted and their frequency during the duration of the lysimeter experiment. Results corresponding to the chemical and physical characteristics of the soils and the greenwaste compost have been reported in **Section 5.3.1.**

Soil sampling within the lysimeter soil profile was done at two depths; 0-10 cm and 10-50 cm. During soil sampling, an auger of 25 mm in diameter was used to collect soil samples that were homogenised through mixing. The holes left after soil sampling were backfilled with similar corresponding soil. The methods for the chemical and physical analyses have been summarised in **Table 5-2**. The physical and chemical characteristics of the two soil types and compost have been reported in **Section 5.3.2.**

Table 5-1 Details of soil and plant analyses conducted during the lysimeter experiment.

Determination	Timing and frequency of analysis
TN_{soil}	• 1st and 2nd cut and end of lysimeter study
TC_{soil}	• Start of study, 1st and 2nd cut and end of lysimeter study
Heavy metals (Cr, Cu, Pb, Ni & Zn)	• Start and end of study
Extractable P	• 1st and 2nd cuts and end of lysimeter study
Total P	• 1st and last cuts
Organic matter	• 1st and last cuts
TN_{plant}	• After each and every ryegrass cut
Dry matter	• After each and every ryegrass cut
Mineral N	• First and last cuts
pH	• First and last cuts
$TN_{effluent}$	• After each STSE irrigation event
NH_4^+ -$N_{effluent}$	• After each STSE irrigation event
NO_3^- -$N_{effluent}$	• After each STSE irrigation event
$P_{effluent}$	• Twice a month during STSE irrigation season
$K_{effluent}$	• Twice a month during STSE irrigation season
STSE conductivity	• After each effluent irrigation event
$pH_{effluent}$	• After each STSE irrigation event
Orthophosphate $_{effluent}$	• After each STSE irrigation event

5.2.2.2 Plant analyses

During the lysimeter experiment, four ryegrass cuts were made. The plant herbage was harvested by cutting using a shear at about 2 cm above the soil surface (Cordovil et al., 2006) and harvested plant material was oven-dried at 60˚C for 48 hours (Evers, 2002). Plant material was processed as reported in **Chapter 4** and analysed for TN_{plant} and TP_{plant} for the determination of ryegrass N and P uptake. Ryegrass N uptake was determined as the product of TN_{plant} and ryegrass dry matter (DM) (Douglas et al., 2003). Similarly, ryegrass P uptake was determined as a product of TP_{plant} and DM yield.

Table 5-2 A summary of the methods used for the analyses of soil, compost and STSE.

Determination	Methods
TN_{soil} and TC_{soil}	• BSI, (BSI, 2000b)
Heavy metals (Cr, Cu, Pb, Ni & Zn)	• US EPA, (1994)
Extractable P	• BSI, (1995)
Total P	• BS EN 13657, (2002)
Organic matter	• BSI, (2000a)
TN_{plant}	• BSI, (2000b).
Soil mineral N	• MAFF, (1986a)
pH	• BSI, (2000c)
CEC	• MAFF (1986b)
Effluent nutrients	• Spectroquant Merck® test kits (Merck (VWR International), Poole, UK)

5.2.3 STSE irrigation

Irrigation was done manually using a watering can with tap water or STSE in lysimeter soils with combined application of compost and STSE and in lysimeters soils amended with STSE alone, ($0_{compost}$ + $100_{effluent}$). Lysimeter soils were irrigated with STSE until the target quantities reported in **Table 5-3** were satisfied. In treatments ($50_{compost}$ + $50_{effluent}$) and ($75_{compost}$ + $25_{effluent}$), after attaining the STSE volumes, the soils were irrigated with tap water. In treatments with no STSE nutrient contribution e.g. ($100_{compost}$ + $0_{effluent}$), lysimeters soils were irrigated with tap water from the start.

Table 5-3 Quantity of greenwaste compost and STSE applied to corresponding nutrient supply combinations to provide 150 kg N ha^{-1} to ryegrass plants.

Compost and STSE combinations (%)	Compost application rate		Projected STSE quantity (mm)
	(t ha^{-1})	(g *lys^{-1})	
$0_{compost} + 100_{effluent}$	0	0	455
$25_{compost} + 75_{effluent}$	2.3	64	341
$50_{compost} + 50_{effluent}$	4.54	128	227
$75_{compost} + 25_{effluent}$	6.82	190	114
$100_{compost} + 0_{effluent}$	9.07	254	0

*Lys stands for lysimeter

STSE and tap water irrigation frequency and irrigation depth were determined based on estimates of evapotranspiration readings taken from an ETgage™ and rain gauge that were installed within the lysimeters experimental yard (**Figure 5.4**). As described in **Chapter 4**, ETgage™ is a modified atmometer. It is a convenient and practical tool for irrigation management. It provides useful information which can be used to accurately estimate the quantity of water to apply and schedule irrigation. Irrigation amount was estimated as the difference between ET and rain. The total nutrient loading rate was calculated using the **Equation 5-1** by Hassanli et al., (2008) and Myers et al., (1999).

$$L_p = \frac{1}{100} * L_w * C_p$$ **Equation 5-1**

Where; L_p = Nutrient loading rate (kg ha^{-1}),

L_w = Amount of applied effluent (mm),

C_p = Average total nutrient concentration in the effluent (mg l^{-1}).

Nutrients in STSE were determined within 5 days of STSE collection. All effluent samples were stored at 5ºC pending analysis. Analysis of $TN_{effluent}$ was done to monitor the quantity of N applied in treatments with effluent N contribution. STSE was regularly analysed for total N, NH_4^+-N, NO_3^--N, P, K, pH, conductivity and Orthophosphate. Nutrient characterisation of the STSE was conducted by employing reactive kits using Spectroquant Merck® test kits (Merck (VWR International), Poole, UK) as described in **Section 3.2.3** of **Chapter 3**. Chemical and physical characteristics of the STSE have been presented in the **Section 5.3 and in Appendix** (**Table B.1-6** and **Table B.1-7**).

Figure 5-4 Rain gauge used to determine precipitation within the lysimeters area for the determination of amount of irrigation.

5.2.4 Leachate analysis

Leachate collection from the lysimeters was done after irrigation or significant rainfall events during the lysimeter study. The first collection of leachate was on 1st June 2011. The frequency and interval of leachate collection was governed by the size of the leachate collection containers so as to avoid over flowing of leachate in the collectors. Upon collection, a leachate subsample of 100 ml was taken, filtered through a 0.45 µm micro pore filter to remove suspended particles. After each collection, leachate samples were analysed for total dissolved N (TDN), ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) using *Burkard Scientific* Segmented Flow Analyser. Leachate samples were stored at -20°C prior to heavy metal analysis. Due to the very low concentration of dissolved heavy metals in leachate samples, heavy metal leachate analyses were conducted at random using the *AAnalyst 800* Atomic Absorption Spectrophotometer (AAS). The amount of NO_3^--N leached was estimated as a percentage of the total amount of N applied according to **Equation 5-2**

$$NO_3^- - N\left(\%\right) = \frac{NO_3^- - N_{leached}}{N_{applied}} \qquad \textbf{Equation 5-2}$$

5.2.5 Statistical analysis

The effects of each treatment and the influence of soil type and compost-effluent combinations on the measured variables during the duration of the lysimeter experiment were assessed by repeated measures analysis of ANOVA and factorial ANOVA (General Linear Models) in Statistica 9.0 to determine significant difference of means. Significantly different levels of treatments were identified using least significant differences at a probability of 0.05 (Fisher's LSD). Probability plots of residuals were used to assess whether or not a data set was approximately normally distributed. Occasionally extreme values were removed during the statistical analyses.

5.3 Results and discussion

5.3.1 Climate

During the lysimeter experiment, recorded rainfall data showed that the amount of rainfall received between March to July 2012 was higher than long-term means (10 year means) over the same time period. In July 2012, monthly rainfall recorded was 114 mm compared to 10 year monthly rainfall for July of 32 mm. **Figure 5-5** shows a comparison of rainfall pattern for the last 10 years to that during the lysimeter experiment (2011/12). In the winter months, apart from December 2011, less rainfall was recorded as compared to the 10 year mean monthly rainfall at the same time. The rest of the weather data has been presented in **Appendix C.3**.

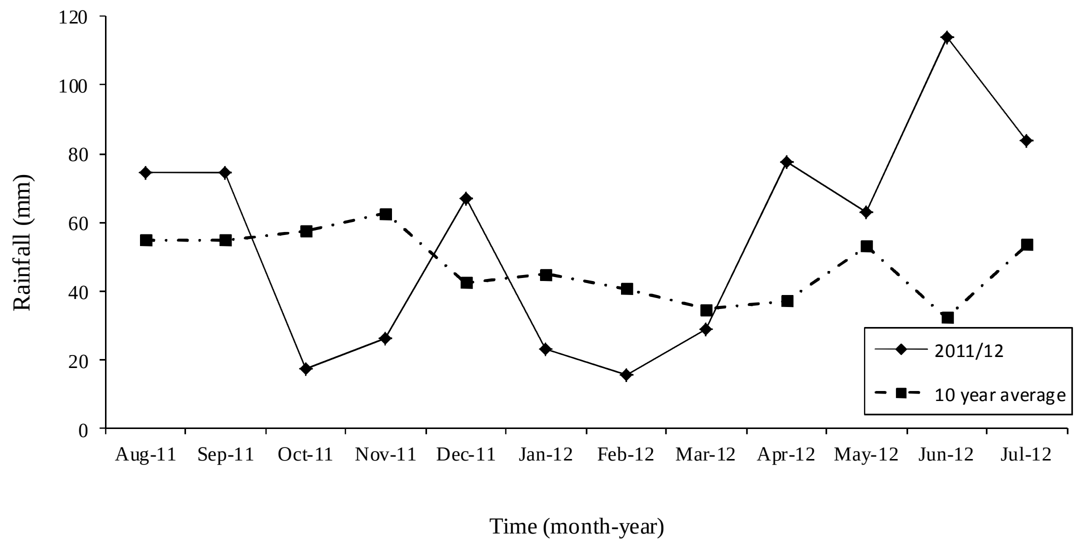


Figure 5-5 Monthly rainfall during the course of the lysimeter experiment (August 2011 to July 2012) and 10 year monthly mean rainfall (Source: Clifton Weather, UK).

The above average rainfall recorded in 2012 affected leachate losses and the quantity of STSE supplied for treatments with higher contribution of effluent e.g. ($0_{compost}$ + $75_{effluent}$) and ($25_{compost}$ + $75_{effluent}$). Due to excessive rainfall experienced in spring and summer of 2012, the projected quantities of STSE required to supply N as presented in **Table 5-4**, were not met for some treatments. As indicated in **Table 5-4** the actual amounts of STSE irrigated for the treatments ($0_{compost}$ + $100_{effluent}$) in clay loam and sandy loam and ($25_{compost}$ + $75_{effluent}$) in the sandy loam did not match the projected required quantities. For these treatments, irrigation with STSE did not supply the required N to the ryegrass plants.

Mean temperatures recorded during the lysimeter study from autumn 2011 to winter 2012 were slightly higher as compared to the 10-year mean monthly temperature for the same time period (**Figure 5-6**). In December 2011 and January 2012, monthly temperature during the lysimeter study was higher by 2.4 and 1°C respectively as compared to the 10-year mean monthly temperature for the same time. In spring and summer 2012, recorded average monthly temperatures were less than the 10-year mean monthly temperature for the same time period (**Figure 5-6**).

Table 5-4 Quantity of greenwaste compost and STSE applied to corresponding nutrient supply combinations to supply 150 kg N ha^{-1} to ryegrass plants.

Treatment combinations (%)	STSE quantity (mm)		
	Projected	Actual (sandy loam)	Actual (clay loam)
$0_{compost} + 100_{effluent}$	455	285	365
$25_{compost} + 75_{effluent}$	341	297	341
$50_{compost} + 50_{effluent}$	227	227	227
$75_{compost} + 25_{effluent}$	114	114	114
$100_{compost} + 0_{effluent}$	0	0	0

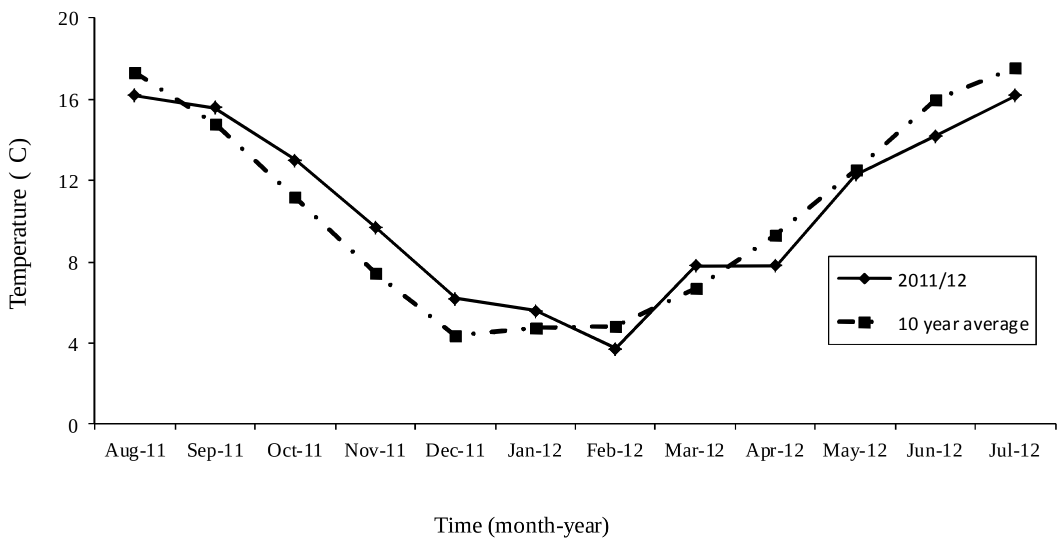


Figure 5-6 Mean monthly temperature during the course of the lysimeter experiment (August 2011 to July 2012 - October 2004) and 10-year average monthly temperature (Source: Clifton weather, Bedfordshire, UK).

5.3.2 Soil, compost and STSE characteristics

STSE used for the study was obtained from Cranfield university sewage treatment plant (CUSTP) and it was analysed for different chemical properties before irrigation to the lysimeters. Using the FAO classification (Ayers and Westcot, 1985) presented in **Chapter 2** (Literature review), the STSE was classified as having none to slight to moderate restriction for agricultural use.

The results of the analysis of STSE during the duration of the lysimeter study are presented in **Table 5-5**. Total N in the STSE was predominantly in the form of NH_4^+-N and NO_3^--N. 71% of the total dissolved N (TDN) was NH_4^+-N and NO_3^--N with the remaining as dissolved organic N. As reported in **Chapter 4**, analyses of STSE showed low to non-detectable levels of heavy metals. Mean dissolved Pb, Cu and Cr were 0.08, 0.005 and 0.016 mg l^{-1} respectively. Ni and Zn were non-detectable in the STSE.

Table 5-5 Chemical and physical properties of STSE for the lysimeter experiment with standard errors of the means (± SEM) (n = 3).

Parameter	STSE	SEM
K (mg l^{-1})	22	1.5
TN (mg l^{-1})	59	0.3
NH_4^+ - N (mg l^{-1})	2.67	1.0
NO_3^--N (mg l^{-1})	39	2.6
P (mg l^{-1})	6.2	1.5
Conductivity (µS cm^{-1})	862	18.9
pH	7.0	0.3
PO_4^{3-} -P (mg l^{-1})	5.9	0.4
DOC (mg l^{-1})	88	6.5

Table 5-6 presents characteristics of the greenwaste compost and soils used in the lysimeter experiment. The sandy loam (*c.*70% sand, *c.* 22% silt and *c.* 8% clay) and clay loam (*c.*32% sand, *c.* 37% silt and *c.* 31% clay) soils were selected in such a way that the textural characteristics were close to that of the soils used in **Chapter 3 & 4**. The concentrations of heavy metals in greenwaste compost were below PAS 100 (BSI, 2005) limits presented in **Chapter 4**.

Table 5-6 Chemical and physical properties of compost and soil prior to the start of the lysimeter experiment (n = 4). Numbers in parenthesis are standard errors of the means (± SEM).

	Sandy loam	Clay loam	Compost
Cu (mg kg^{-1})	UD*	32 (1.2)	UD*
Cr (mg kg^{-1})	30 (0.9)	34 (1.9)	19 (0.0)
Ni (mg kg^{-1})	10 (0.4)	24 (3.5)	10 (0.4)
Pb (mg kg^{-1})	47 (2.1)	234 (4.6)	128 (2.2)
Zn (mg kg^{-1})	49 (2.9)	135 (1.7)	160 (3.1)
K (g kg^{-1})	1.6 (0.1)	4.4	12.9 (0.2)
CEC (cmol+ kg^{-1})	10.6 (0.2)**	15.6 (1.1)**	100 (2.6)***
TN (%)	0.2 (0)	0.1 (0)	1.8 (0.02)
TC (%)	2.9 (0.07)	1.6 (0.02)	20.5 (0.2)
NO_3^--N (mg kg^{-1})	18.3 (0.4)	24.2 (0.03)	517 (2.0)
NH_4^+-N (mg kg^{-1})	0.00	0.6 (0)	2.54 (0.3)
TP (mg kg^{-1})	514 (10.4)	473 (5.3)	2.1 (0.04)
pH	7.7 (0.01)	6.9 (0.03)	7.87 (0.01)
SOM (%)	3.9 (0.03)	5.7 (0.1)	36.4 (1.1)

UD* = undetectable

** Determination using Barium chloride

*** Determined using Ammonium acetate

5.3.3 Nutrient leaching

5.3.3.1 Nitrogen

During the duration of the lysimeter experiment, leachate samples were collected 16 times. At the same time, leachate collection was governed by the principle of waiting as much as possible until all collectors had leachate. Statistical analysis of cumulative NO_3^--N in leachate in both soils started from third leachate samples as the data for the first and second leachate samples could not be normalised due to low to non-detectable levels of NO_3^--N in the leachate samples.

Figure 5-7 and Figure 5-8 show the effect of the combinations of compost and STSE and soil types with time on cumulative NO_3^--N leaching during the duration of the lysimeter study (p = 0.03). In the sandy loam (**Figure 5-7**), the combinations of compost and STSE did not significantly differ from each other in terms of cumulative NO_3^--N leaching. By the end of the lysimeter study in the sandy loam, 1.3 kg NO_3^--N ha^{-1} was lost from the treatment ($50_{compost}$+$50_{effluent}$). For the other combinations of compost and STSE, cumulative NO_3^--N losses were 1.05, 0.77, 0.76 and 0.54 kg NO_3^--N ha^{-1} ($25_{compost}$+$75_{effluent}$), ($75_{compost}$+$25_{effluent}$), ($0_{compost}$+$100_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively. Cumulative NO_3^--N loss through leaching was significantly influenced by the soil types.

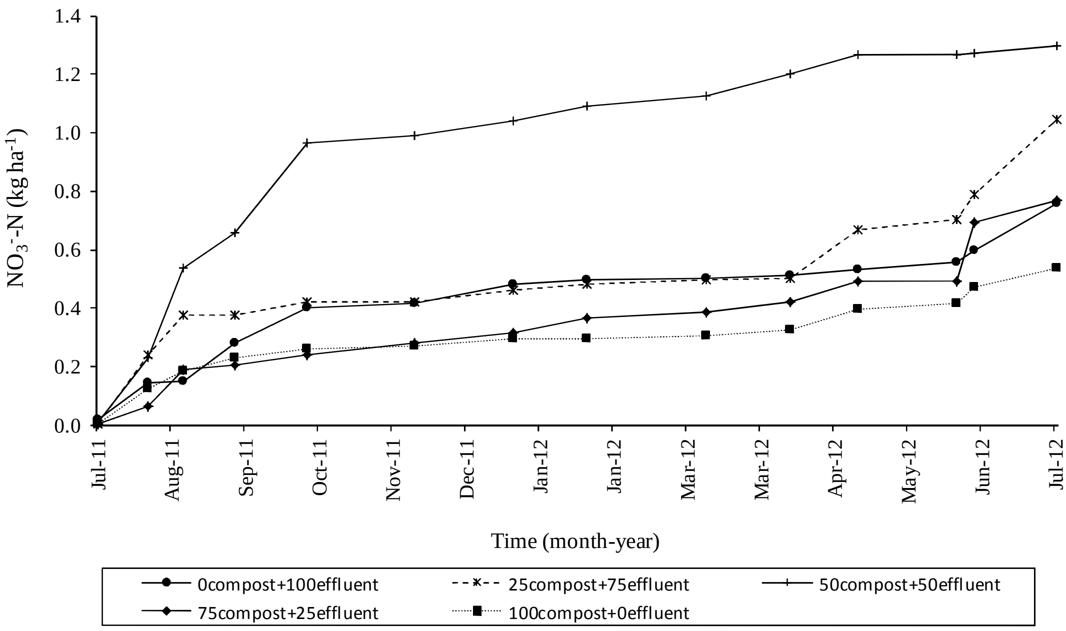


Figure 5-7 Cumulative mean NO_3^--N leaching losses (kg ha^{-1}) with time for combinations of compost and STSE in sandy loam (p = 0.03).

The rate of cumulative NO_3^--N loss in the sandy loam for all combinations of compost and STSE was related to the volume of leachate collected. The volume of leachate collected corresponded to the amount of rainfall registered during this time. In winter months, the loss of NO_3^--N due to leaching was minimal. In August and September 2011, average monthly rainfall was 75 mm while from May to July 2012, rainfall ranged from 63 to 114 mm (**Section 5.2.1**). In summer and autumn months, higher evapotranspiration losses resulted in higher irrigation frequency and the amount of STSE irrigated was higher as well.

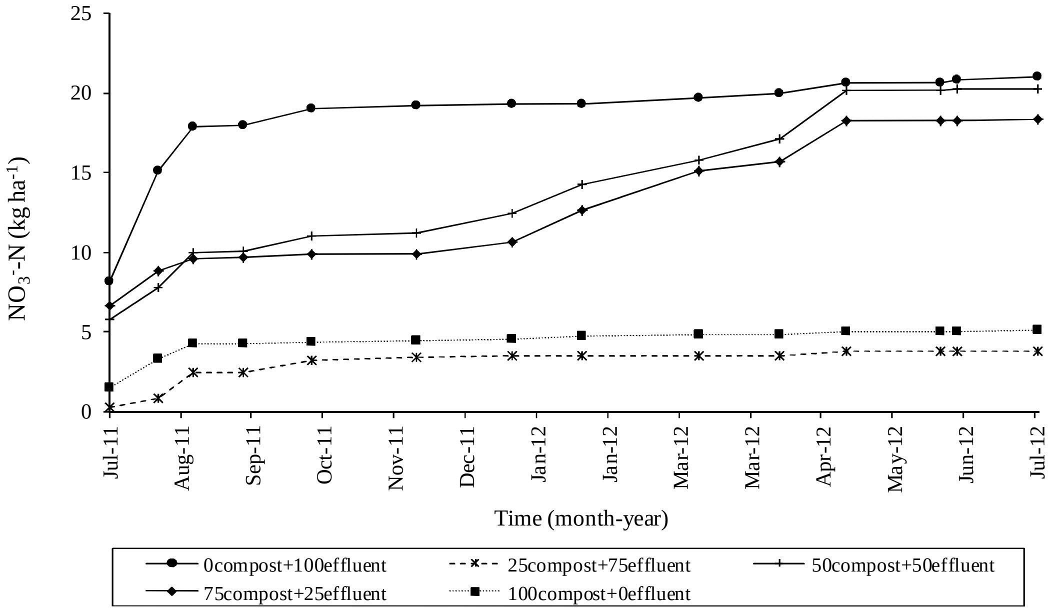
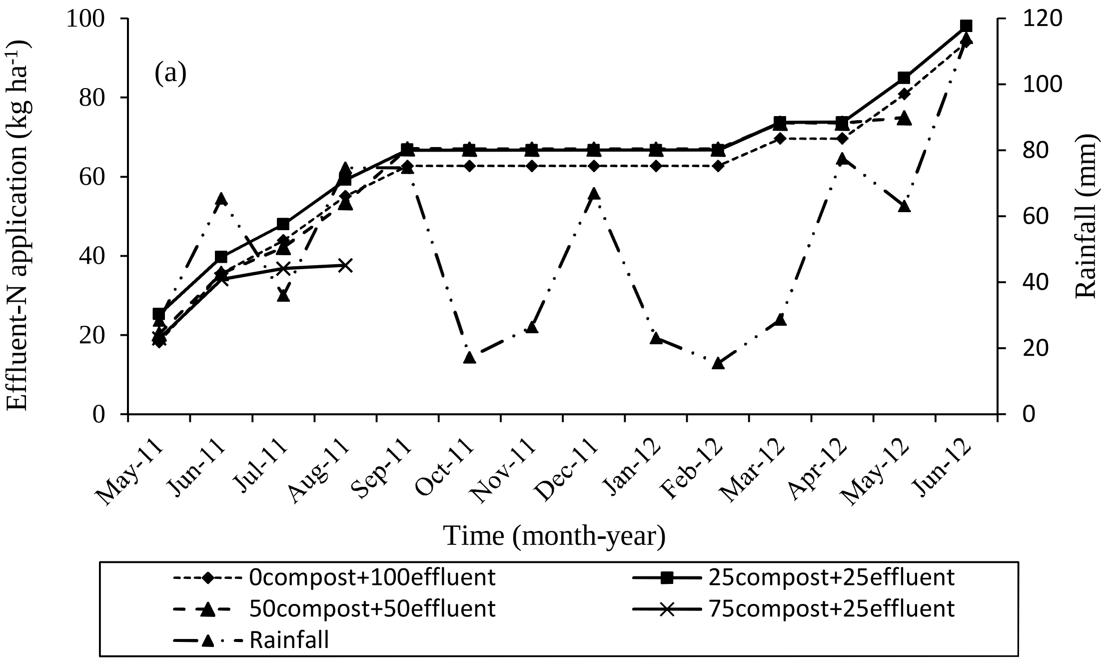


Figure 5-8 Cumulative mean NO_3^--N leaching losses (kg ha^{-1}) with time for combinations of compost and STSE in clay loam (p = 0.03).

In the clay loam, cumulative loss of NO_3^--N was significantly higher in treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) than the treatments, ($100_{compost}$+$0_{effluent}$) and ($25_{compost}$+$75_{effluent}$). At the end of the study, cumulative NO_3^--N losses were 21, 3.8, 20.3, 18.4 and 5.1 kg NO_3^--N ha^{-1} for the treatment ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$), ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively (**Figure 5-8**).

Statistical analysis of cumulative NO_3^--N in leachate indicated that, overall the combinations of compost and STSE did significantly influence cumulative NO_3^--N loss from the lysimeters (p = 0.01). In all combinations of compost and STSE, cumulative NO_3^--N loss peaked in August 2011 and increased at a slow rate apart from the treatments ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$). For the treatments ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) in the clay loam, the cumulative loss of NO_3^--N increased progressively until the end of the experiment. **Figure 5-9** shows cumulative application of STSE-N during the lysimeter experiment for the combinations of compost and STSE. The period that corresponded with peak cumulative losses of NO_3^--N was in the spring of 2012. During this period, the quantities of STSE applied to combinations of compost and STSE was also higher.



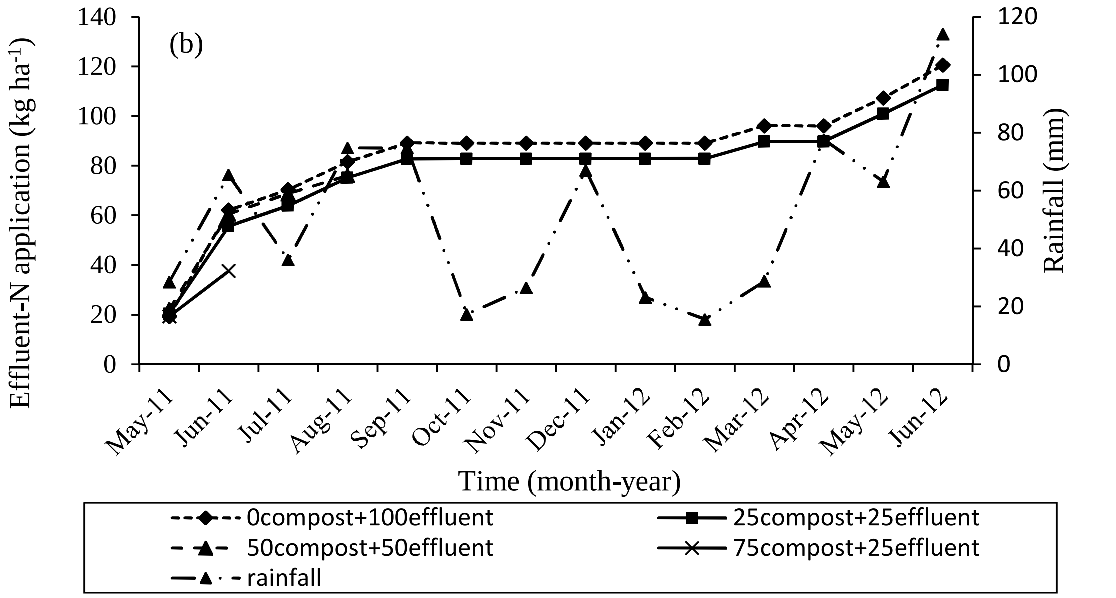


Figure 5-9 Cumulative application of N through STSE in treatments with effluent-N contribution in a) sandy loam and b) clay loam

In relation to the soil types, cumulative mean NO_3^--N loss was significantly lower in the sandy loam as compared to the clay loam soil. When averaged for the combinations of compost and STSE, cumulative mean NO_3^--N loss was 0.5 kg NO_3^--N ha^{-1} in the sandy loam and 10.6 kg NO_3^--N ha^{-1} in the clay loam soil. Availability of N in sandy loam soil reported in **Chapter 3** was significantly lower as compared to the clay loam soil due to low net N mineralisation in the sandy loam soil. Low net N mineralisation reduced susceptibility of N to leach in the sandy loam soil.

Table 5-7 summarises the amount of NO_3^--N leached from the two soil types expressed as a percentage of the total N applied for the combinations of compost and STSE. It should be noted that some of the NO_3^--N leached was possibly from the soil's native NO_3^--N which could not be accurately estimated. For the treatment ($0_{compost}+100_{effluent}$) in the clay loam, *c.* 21% of the total applied N was lost as NO_3^--N leaching. For the same treatment in the sandy loam soil, only *c.* 0.5% was lost. The percentage of total N lost as NO_3^--N was highest in the clay loam and decreased with increasing compost contribution in treatments with compost-STSE combinations. In both soil types, the lowest losses were in treatments with compost alone, ($100_{compost}+0_{effluent}$). The slow release of nutrients from compost may lessen adverse environmental effects from N leaching (Chang and Janzen, 1996).

Table 5-7 NO_3^--N leaching expressed as a percentage of actual total N supplied for the combinations of compost and STSE.

Compost and treated sewage effluent combination	NO_3^--N leached (%)	
	Sandy loam	Clay loam
$0_{compost}+100_{effluent}$	0.51	20.51
$25_{compost}+75_{effluent}$	0.34	7.88
$50_{compost}+50_{effluent}$	0.70	8.31
$75_{compost}+25_{effluent}$	0.21	7.09
$100_{compost}+0_{effluent}$	0.20	3.02

Figure 5-10 and Figure 5-11 show how the concentration of NO_3^--N in the leachate varied over the course of the lysimeter experiment in the sandy loam and clay loam soils respectively. The three way interaction of time, soil type and compost-effluent combination was significantly different ($p = 0.00$). The concentration of NO_3^--N during the duration of the study was significantly influenced by the soil types and combinations of compost and STSE. NO_3^--N concentration (for the five N compost-effluent combinations) was significantly higher ($p = 0.00$) in the clay loam as compared to the sandy loam soil. In the clay loam, NO_3^--N concentration was 11.48 mg l^{-1} while in the sandy loam, it was 1.75 mg l^{-1}. Despite low mean NO_3^--N concentration in the sandy loam, peaks of higher concentrations were observed (**Figure 5-10**). The treatment ($25_{compost}$+$75_{effluent}$) registered peak NO_3^--N concentration on 9th August 2011, 28th September 2011 and 21st December 2011 of 11.4, *c.* 10 and 11.1 mg l^{-1} respectively. Similarly higher peaks were observed for the treatment ($50_{compost}$+$75_{effluent}$) though the NO_3^--N concentration was below 10 mg l^{-1}. The drinking water NO_3^--N standard threshold is < 10 mg l^{-1} (Thompson et al., 2007; Thomas et al., 2006) while in the EU and UK, the drinking water standard is 50 mg NO_3^--N l^{-1}. In this research, the drinking water standard of 10 mg NO_3^--N l^{-1} will be used.

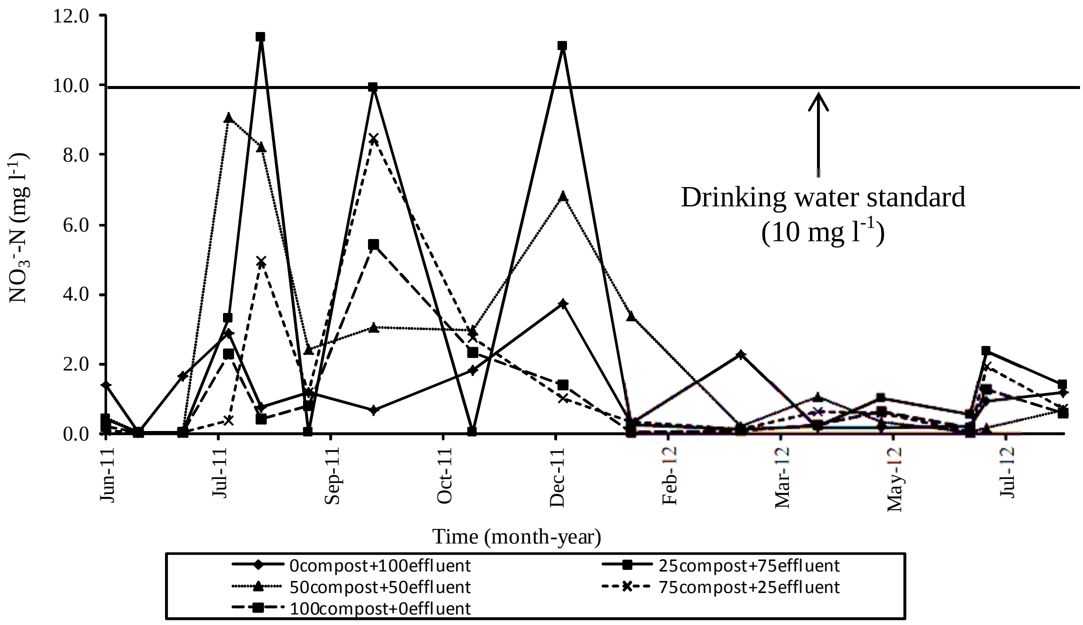


Figure 5-10 Mean concentration of NO_3^--N in leachate collected from the lysimeter experiment in sandy loam (p = 0.00).

In the clay loam soil (**Figure 5-11**), peak concentrations of NO_3^--N > 10 mg l^{-1} were largely observed in 2011 mostly for treatments with effluent N contribution. The highest concentration was for the treatment ($0_{compost}$+$100_{effluent}$). For this treatment, during the study duration NO_3^--N concentration ranged from 0.1 to 59 mg l^{-1} while for the ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) treatments, the concentration ranged from 0.1 to *c.* 51 mg l^{-1}. The average concentration of NO_3^--N decreased significantly with time (p = 0.00). In 2012 despite the recorded higher rainfall, the concentration of NO_3^--N was low.

STSE irrigation on soils amended with compost or in treatments with STSE alone increased the concentration of NO_3^--N in leachate. This can explain the higher concentration of NO_3^--N in the clay loam soil for the treatments ($0_{compost}$+$100_{effluent}$) ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$). Despite being one of the primary sources of N to plants, the chemical characteristics of NO_3^--N make it susceptible to leaching through the soil profile and into the shallow groundwater by the infiltrating water (Basso and Ritchie, 2005). NO_3^--N in excess of 10 mg l^{-1} is a threat to the quality of drinking water.

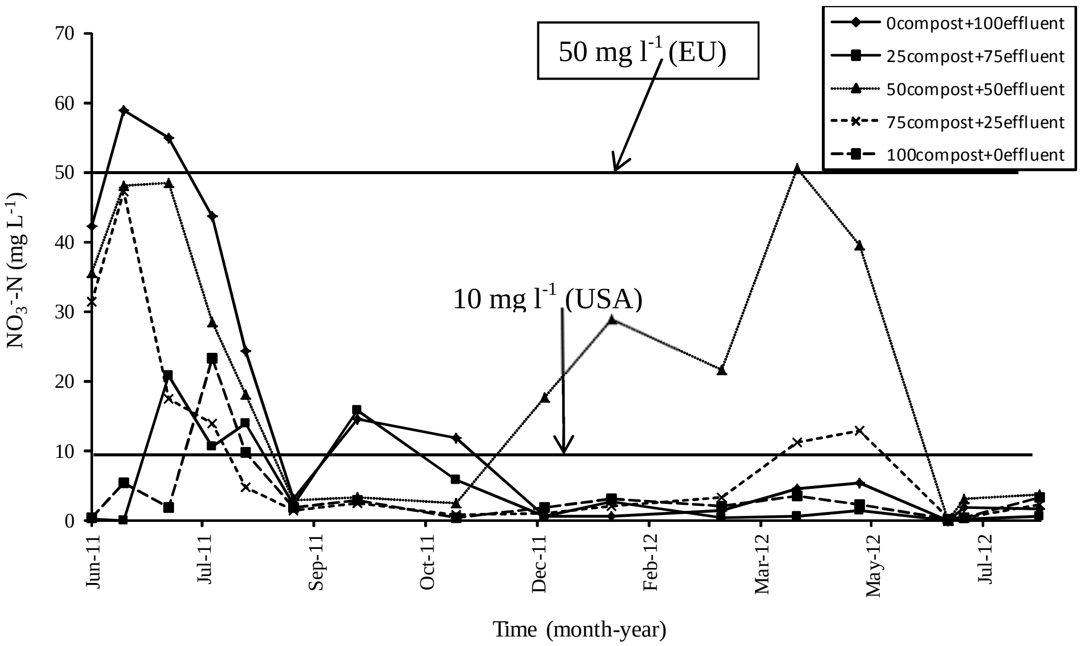


Figure 5-11 Mean concentration of NO_3^--N in leachate collected from the lysimeter experiment in clay loam (p = 0.00).

The concentration of NH_4^+-N in leachate was not significantly affected by soil type and combinations of compost and STSE. But with time, concentrations of NH_4^+-N were significantly different. Overall peaks of concentration of NH_4^+-N were observed during the study. In the clay loam soil, highest peak concentration of NH_4^+-N (for the combinations of compost and STSE) was observed in leachate samples obtained in June 2011 (**Figure 5-12)**. *Post-hoc* analysis in Statistica revealed that the source of this higher peak was the treatment ($0_{compost}$+$100_{effluent}$). **Figure 5-12** shows the interaction of soil type and leachate sampling time on mean NH_4^+-N concentration.

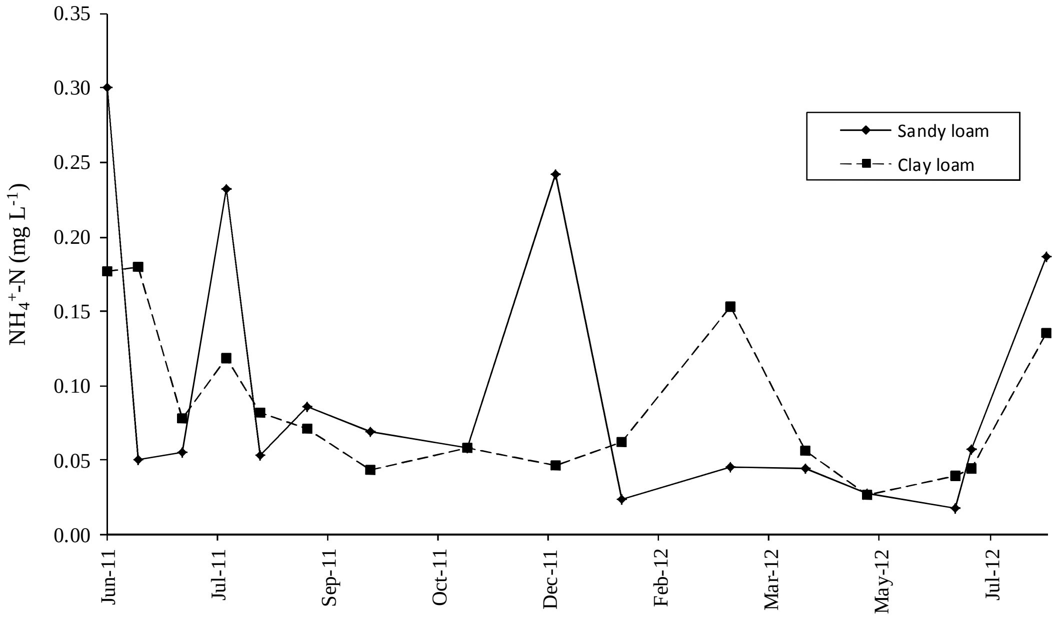


Figure 5-12 Mean concentration of NH_4^+-N in leachate collected from lysimeters experiment in clay loam and sandy loam ($p = 0.02$).

Overall, the mean concentration of NH_4^+-N for the various combinations of compost and STSE was not significantly different across the soil types. The concentration of NH_4^+-N in leachate ranged from 0.05 to 0.14 mg l^{-1} when averaged over the sampling times. The process of nitrification was rapid resulting in NH_4^+-N conversion to NO_3^--N hence low concentrations of NH_4^+-N in leachate. Above all, the average concentration of NH_4^+-N in STSE across all treatment combinations was 2.7 mg l^{-1}.

Total dissolved N (TDN) in leachate was determined at the same time as NO_3^--N, NH_4^+-N and phosphate. TDN is made up of dissolved organic N, NO_2^--N, NH_4^+-N and NO_3^--N. Repeated measures ANOVA on cumulative TDN for the leachate samples showed significant effluence of soil type on TDN losses from the soil. Loss of TDN was significantly higher ($p < 0.05$) in the clay loam soil (10.86 kg ha^{-1}) than in the sandy loam (0.88 kg ha^{-1}) when averaged for all the combinations of compost and STSE and sampling times.

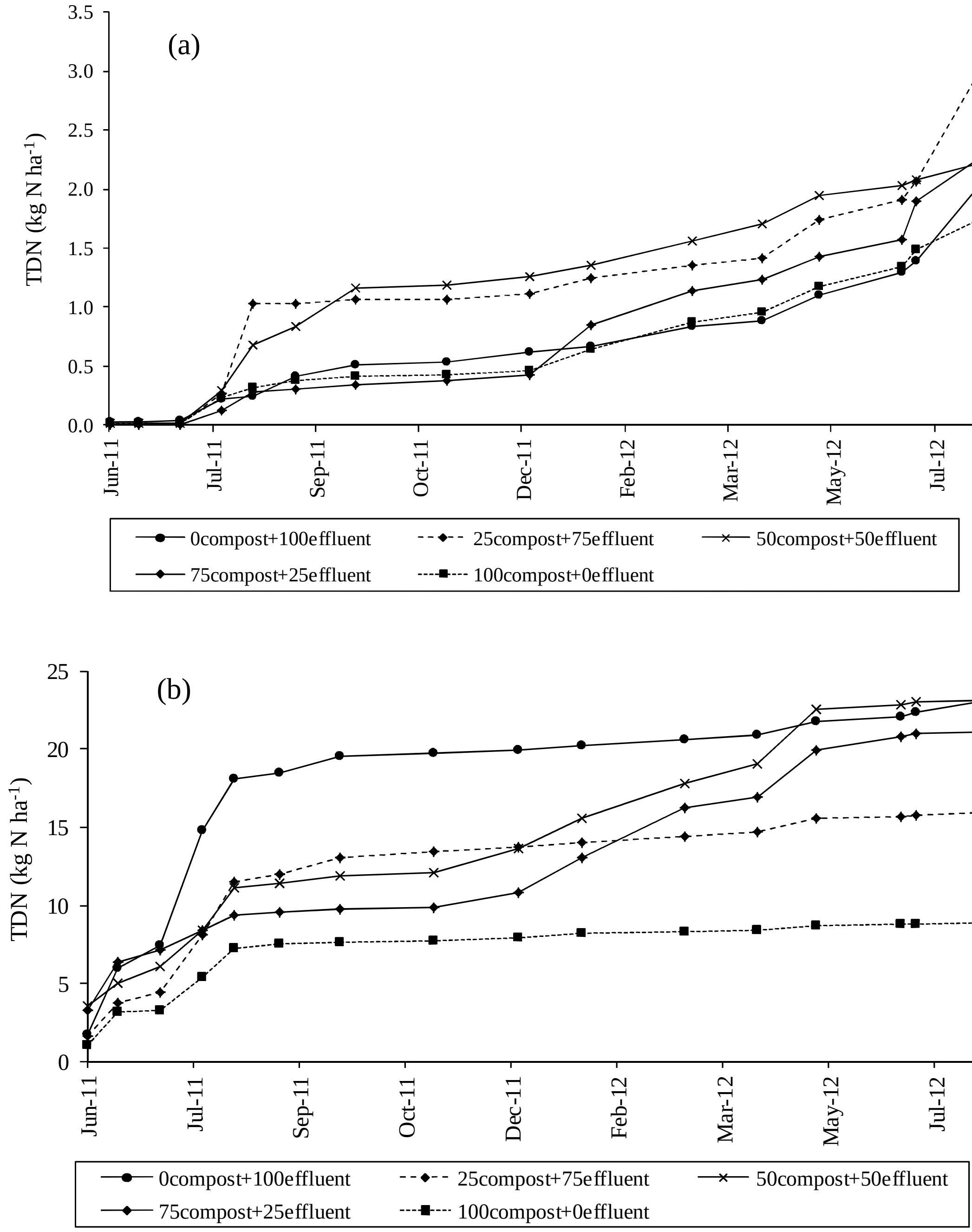


Figure 5-13 Cumulative TDN losses from combinations of compost and STSE in a) sandy loam and b) clay loam soil during the lysimeter experiment (p = 0.05).

Figure 5-13 shows TDN (averaged for the two soil types) as influenced by the interaction of leachate collection time, the combinations of compost and STSE and the soil types (p = 0.05). The highest cumulative TDN losses were in treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) while the treatment with compost alone, ($100_{compost}+0_{effluent}$) had one of the lowest losses of TDN in clay loam soil (**Figure 5-13b**). Cumulative TDN loss was 8.9 kg ha^{-1} ($p < 0.05$) for ($100_{compost}+0_{effluent}$) treatment, while for ($0_{compost}+100_{effluent}$), ($50_{compost}+50_{effluent}$) and ($75_{compost}+25_{effluent}$) it was 23.1, 23.1 and 21.1 kg ha^{-1} ($p > 0.05$) respectively. In the sandy loam, cumulative TDN losses were not significantly different for all the combinations of compost and STSE for all the sampling times.

TDN losses were significantly influenced by the interaction of the sampling times and the soil types. The loss of TDN through leaching with time was significantly higher in the clay loam soil as compared to the sandy loam soil. By the end of the study, in the sandy loam and the clay loam soils, the average cumulative TDN losses were 16.26 and 2.67 kg ha^{-1} respectively (**Figure 5-14**). Availability of N in leachate signified that either the ryegrass plants were not efficient in utilising N or that rate of N mineralisation was higher such that N was susceptible to leaching.

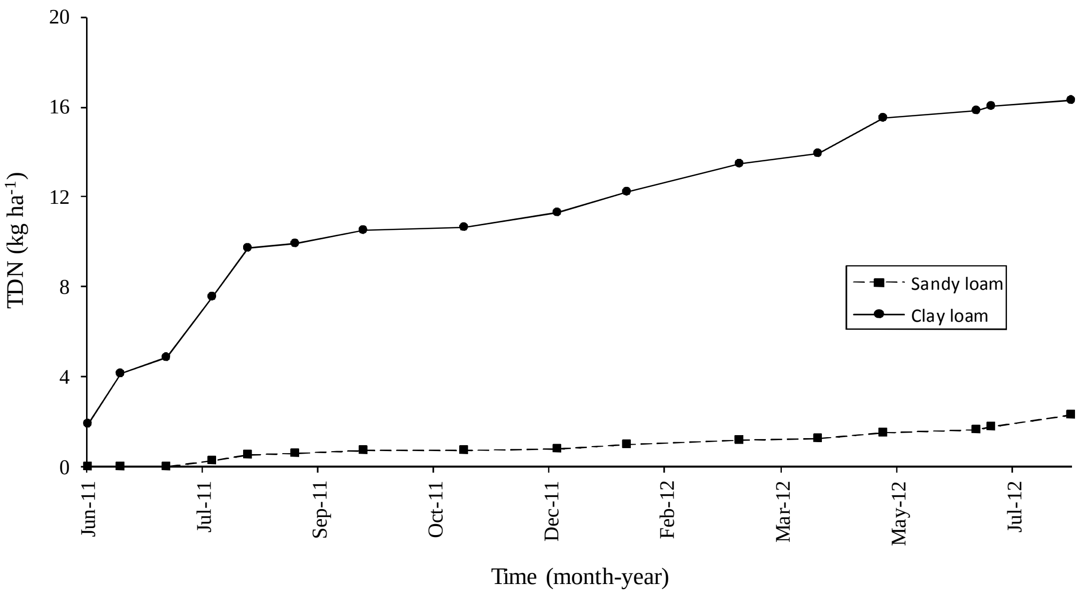


Figure 5-14 Cumulative mean TDN loss from combinations of compost and STSE (for the compost-effluent combinations) in the sandy loam and the clay loam soils during the lysimeter study (n = 6 and p = 0.00).

Analysis of the contribution of NO_3^--N in cumulative TDN showed that 45% and 80% of TDN lost in the sandy loam and the clay loam soils respectively were in the form of

NO_3^--N. This implied that in the sandy loam, TDN was largely in the form of dissolved organic N as the concentrations of NO_2^--N and NH_4^+-N were very low in the leachate.

Figure 5-15 shows the mean contribution of NO_3^--N in TDN for the combinations of compost and STSE in the sandy loam and clay loam soils. The estimated contribution of NO_3^--N in TDN due to the interaction of soil type and compost-STSE combinations was not significantly different (P = 0.24). However, the higher content of NO_3^--N in TDN for combinations of compost and STSE in the clay loam reinforces the threat to water quality. The proportion of NO_3^--N in TDN was significantly higher in treatment combinations of compost and STSE in the clay loam soil. The lowest proportion of NO_3^--N in TDN was for the treatment ($100_{compost}$+$0_{effluent}$) in the clay loam. As reported in **Chapter 3**, net N mineralisation was higher in treatments with higher N contribution from effluent. Availability of NO_3^--N was therefore enhanced with the presences of effluent, thereby increasing the susceptibility of NO_3^--N to leaching and higher proportion in leached total dissolved N. The possibility of organic N in leachate was higher in all combinations of compost and STSE as leachate was not fully made up of NO_3^--N.

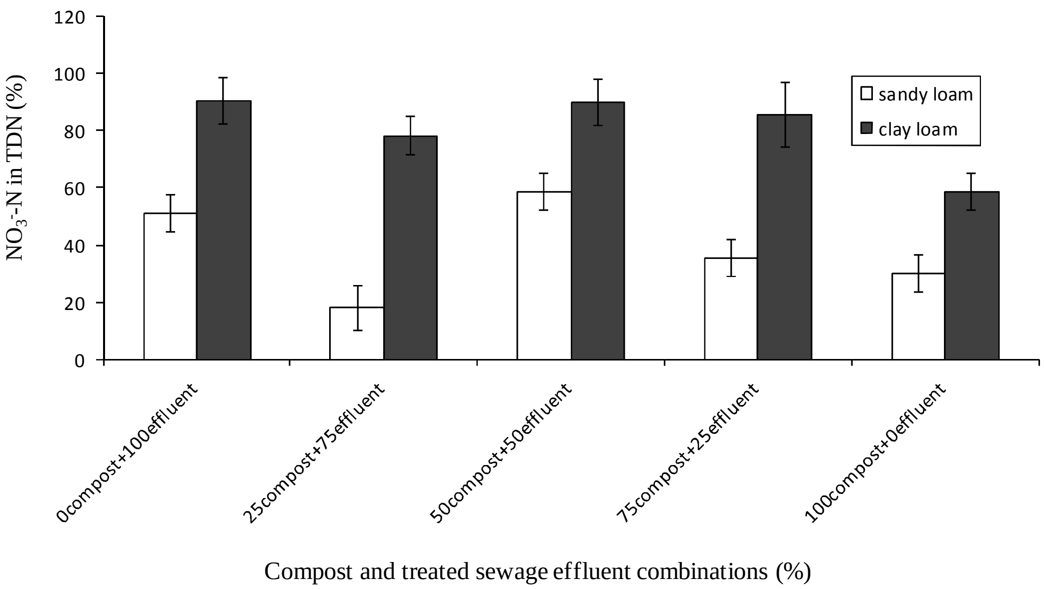


Figure 5-15 Contribution of NO_3^--N in cumulative TDN for the combinations of compost and STSE in the sandy loam and the clay loam soil (p = 0.24). Error bars represent ± SEM.

5.3.3.2 Phosphate-Phosphorous

Phosphate analysis in leachate was conducted at the same time as the other leachate analyses from 1st June 2011 to 30th July 2012. Repeated measures ANOVA showed non-significant effect of soil type and compost-effluent combinations on cumulative PO_4^{3-}-P in leachate during the study period. Cumulative PO_4^{3-}-P loss was minimal for all combinations of compost and STSE in both soil types. In the clay loam at the end of the lysimeter experiment, cumulative loss of P through leaching were 0.047, 0.140 and 0.034 kg P ha^{-1} for the treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($100_{compost}$+$0_{effluent}$), respectively while in the sandy loam it was 0.042, 0.058 and 0.018 kg P ha^{-1} for the same treatments. Susceptibility of PO_4^{3-}-P to leach is minimal despite that many soils have large reserves of P as most often only one per cent is available to crops (Shenoy and Kalagudi, 2005).

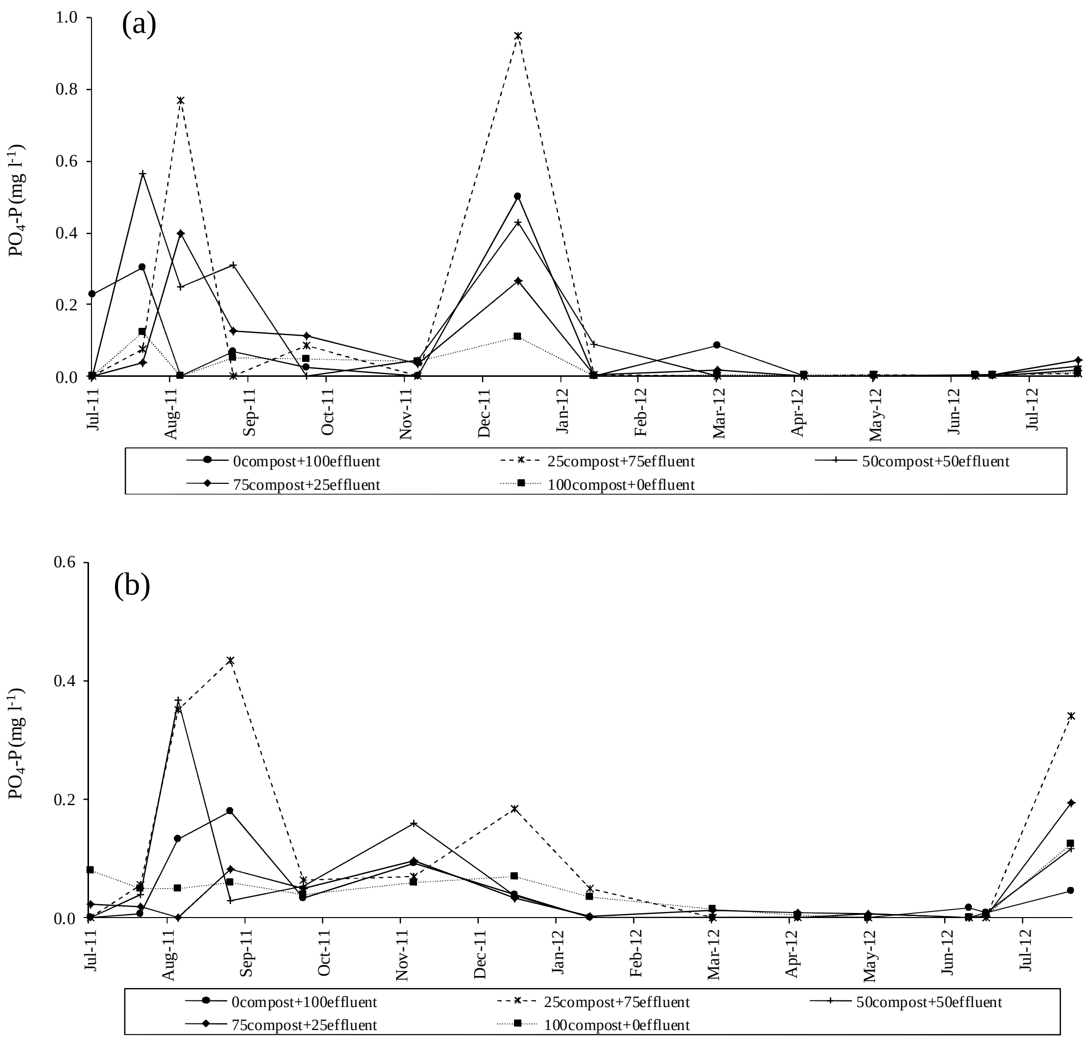


Figure 5-16 Concentration of PO_4^{3-}-P in leachate during the duration of the lysimeter study for the combinations of compost and STSE ($p = 0.07$) in a) sandy loam and b) clay loam soil.

Assessment of the threat of P leaching to the environment was carried out by analysing the concentration of PO_4^{3-}-P in leachate collected from the lysimeters. **Figure 5-16** presents the analysis of PO_4^{3-}-P concentration in leachate. It showed that the concentration PO_4^{3-}-P in leachate was significantly influenced by the combinations of compost and STSE. However, despite the significant effect of the combinations of compost and STSE, the differences of mean PO_4^{3-}-P concentration in leachate amongst the various combinations of compost and STSE were small with a range of 0.04 to 0.12 mg l^{-1} (mean across the soil types). But potential for P losses depends on the amount of P that can be rapidly released into the soil solution between the treatments (Weaver et al., 1988). Excessive losses of P contribute to eutrophication of water bodies. Apart from effluent samples collected in spring 2012, the concentration of PO_4^{3-}-P was above the limit that can stimulate growth of algae and other aquatic plants in water bodies (Hinesly and Jones, 1990).

5.3.4 Ryegrass production

5.3.4.1 Ryegrass dry matter

During the lysimeter experiment, four ryegrass cuts were made. Ryegrass cutting interval was governed by the growth of ryegrass following the reasoning explained in **Chapter 4**. Ryegrass cutting intervals were 71, 144, 353 and 476 days after germination. Longer cutting interval (for the third ryegrass cut) was associated with the winter season with low temperatures and reduced day light length limiting ryegrass growth.

The results of DM yield analysis have been presented in **Figure 5-17** and **Figure 5-19**. Repeated measures ANOVA analysis of DM yield for all the cuts made during the study showed that mean DM yield was significantly influenced by the main treatments (combinations of compost and STSE and soil type). The mean DM yield per cut was significantly higher in the clay loam as compared with the sandy loam soil. Mean DM yield per cut was 2943 and 3794 kg DM ha^{-1} for treatments in the sandy loam and clay loam soils respectively. Clay loam soil was more fertile as compared to the sandy loam soil. From **Section 5.3.1**, it can be observed that the clay loam was rich in organic matter; with *c.* 5.7% as compared to *c.* 4% OM in the sandy loam. In relation to the combinations of compost and STSE, irrespective of the soil types, mean DM yield per cut was significantly higher for treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) as compared to the rest of the compost-STSE combinations. Ryegrass DM yield per cut was 3596 and 3441 kg DM ha^{-1} for ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) treatments respectively.

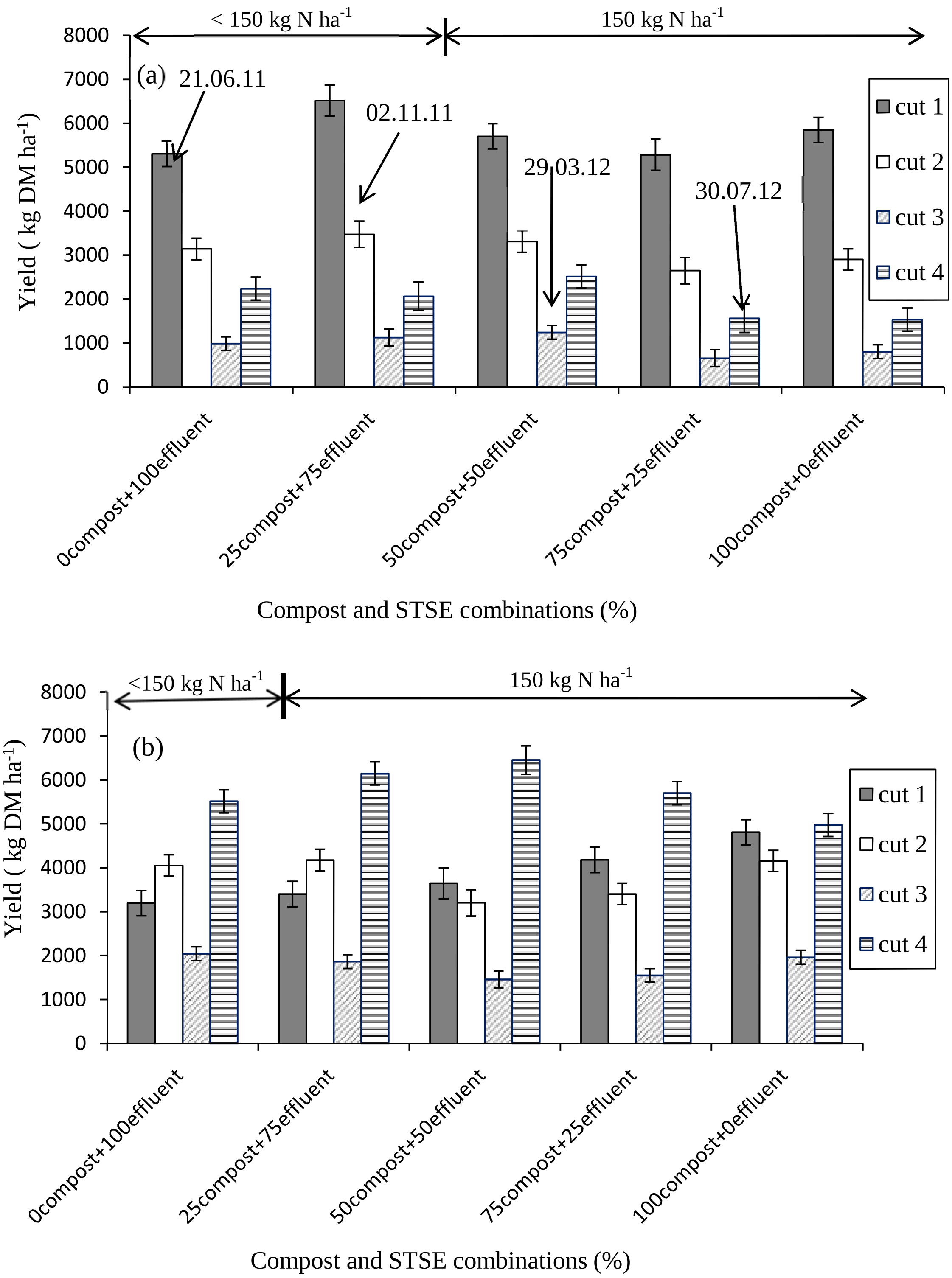


Figure 5-17 DM yield for ryegrass cuts made for the combinations of compost and STSE in a) sandy loam and b) clay loam ($p = 0.054$). Error bars represent ± SEM.

Figure 5-17 shows the overall non-significant effect of the interaction of the combinations of compost and STSE and soil types, ryegrass cuts on DM yield.

However, one notable observation in relation to the timing of the ryegrass cuts was the reduced ryegrass DM yield for all combinations of compost and effluent in both soil types for the third cut (**Figure 5-17)**. The third cut was made on 29 March 2012. This was after the winter seasons while the first and last cuts were made on 21 June 2011 and 30 July 2012 respectively. **Figure 5-18** shows the relationship between timing of ryegrass cuts, cumulative effluent-N application and rainfall recorded during the lysimeter experiment.

Apart from the influence of the environmental on ryegrass DM yield, N mineralisation and immobilisation patterns affected DM production. In the clay loam for example, for the treatments ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$), for the first two cuts, DM yield increased before declining for the third cut (made just after the winter season). While for the rest of the treatments, DM yield declined for the first three cuts before increasing for the last cut. These are also treatments with large contributions of compost (($50_{compost}$+$50_{effluent}$), ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$)). In **Chapter 3**, it was concluded that with increased compost contribution, N mineralisation reduced in the clay loam which affected availability of N to plants.

Similarly in the sandy loam soil, DM yield for all the combinations of compost and STSE declined despite a slight increase ($p < 0.05$) for the last cut DM. This trend signified limited availability of N apart from the environmental factor which was in agreement with observations made for the sandy loam in **Chapter 3**.

Availability of soil NO_3^--N for plant uptake is severely limited at lower temperatures (Tisdale et al., 1990). Limited availability of mineral N (largely NO_3^--N) reduced susceptibility of NO_3^--N to leaching. As presented in **Figure 5-18,** during this period (winter and early spring 2012), cumulative effluent-N application was constant indicating that no STSE was irrigated. This explains also why NO_3^--N in leachate was minimal during this period (**Figure 5-11**). Mean DM yield per cut was significantly higher in the clay loam soil as compared to the sandy loam. In relation to the combinations of compost and STSE, mean DM yield per cut was significantly higher for the treatment ($25_{compost}$+$75_{effluent}$) of 3656 kg DM ha^{-1} (mean for the two soils) as compared to the rest of the combinations of compost and STSE.

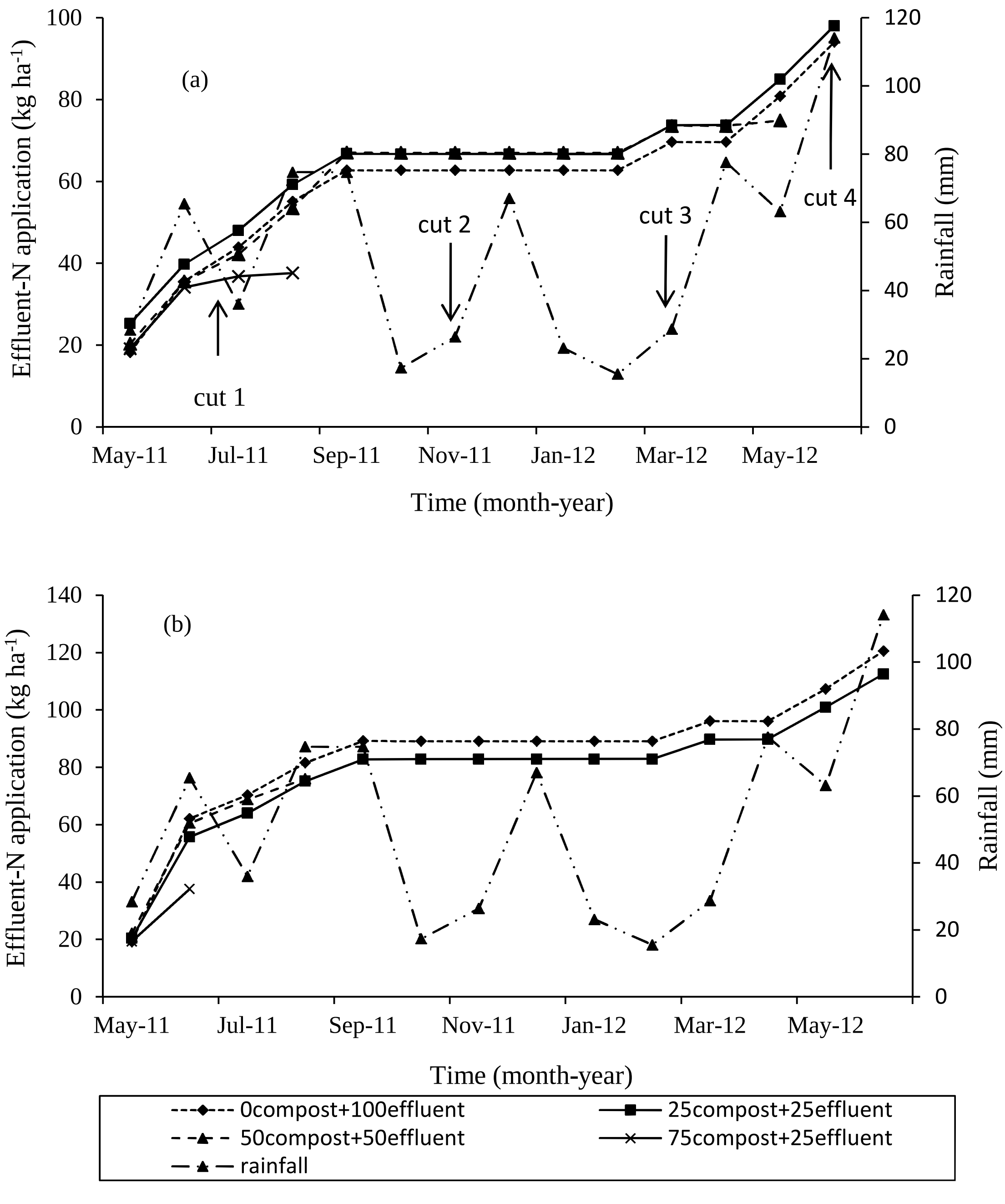


Figure 5-18 Relationship between cumulative effluent nitrogen application, rainfall and timing of ryegrass cuts times during the lysimeter experiment in a) sandy loam and b) clay loam soils.

Figure 5-19 demonstrates the effect of the interaction of soil type and compost-STSE combinations on total DM yield. Total yield per application of 150 kg N ha^{-1} was calculated as a sum of ryegrass DM yields for four ryegrass cuts. Total DM yield was significantly influenced by the soil types and the combinations of compost and STSE. In the clay loam, total DM yield increased significantly by 29% as compared to the sandy loam. Total DM yield (mean of the two soil types) was significantly higher for the treatment ($25_{compost}$+$75_{effluent}$) as compared to all the other combinations of compost and STSE treatments. Total DM yield for the treatment ($0_{compost}$+$100_{effluent}$) was significantly lower than for treatment ($25_{compost}$+$75_{effluent}$) despite the treatment ($0_{compost}$+$100_{effluent}$) supplying more readily available N through STSE. Total DM yield was 14583 kg DM ha^{-1} for ($25_{compost}$+$75_{effluent}$) treatment while for ($0_{compost}$+$100_{effluent}$), it was 13237 kg ha^{-1} ($p < 0.05$). However, according to **Section 5.3.1** (**Table 5-3**), N application rate of 150 kg N ha^{-1} for the treatment ($0_{compost}$+$100_{effluent}$) in the clay loam was not attained, instead only 63% of the required total N was provided to the ryegrass. This shortfall would have influenced ryegrass DM yield for treatment ($0_{compost}$+$100_{effluent}$).

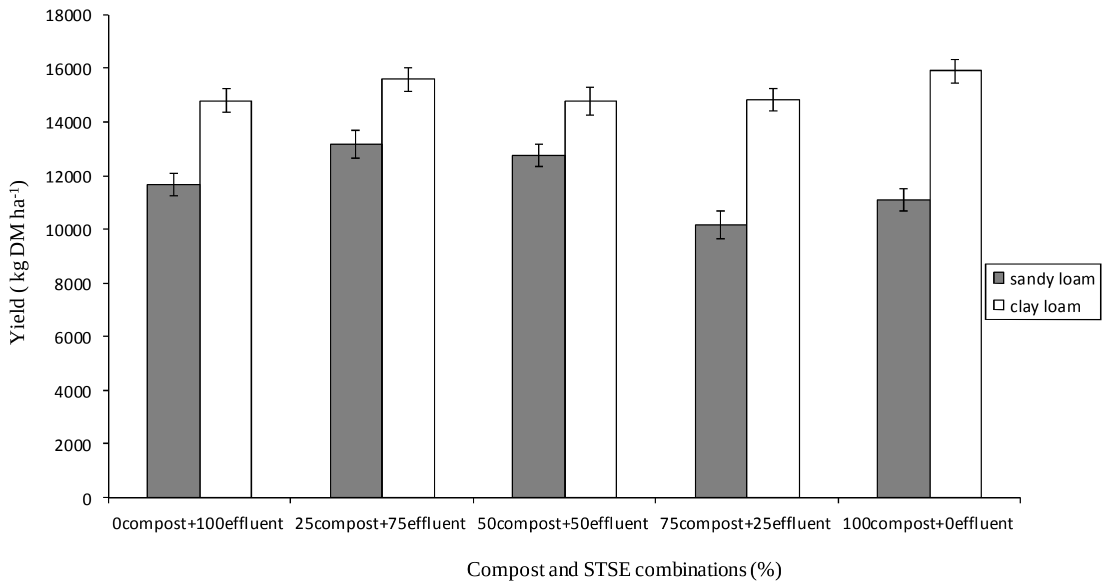


Figure 5-19 Influence of the combinations of compost and STSE on total DM yield for sandy loam and clay loam soil ($p = 0.02$). Error bars represent ± SEM.

5.3.4.2 Nitrogen in plant harvested material

Nitrogen in plant material (TN_{plant}) was assessed in the ryegrass from all the four cuts made. Statistically, TN_{plant} was significantly influenced by the soil types ($p < 0.05$) while non-significant differences were observed for the combinations of compost and STSE. In the clay loam (for the compost-effluent combinations), mean TN_{plant} increased by 22% as compared to the sandy loam soil. The clay loam soil was more fertile as compared to the sandy loam soil with a C/N ratio of 11 and *c.* 15 for the sandy loam. A

C/N ratio of 15 is the critical limit classified by Springob and Kirchmann (2003) as separating soils with higher or lower N release.

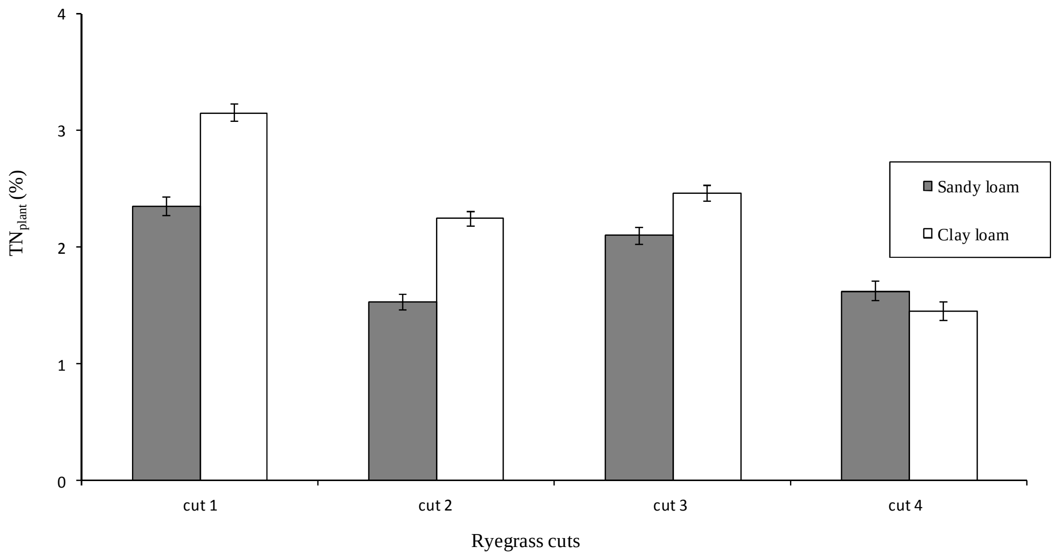


Figure 5-20 Influence of the interaction of soil type and ryegrass cutting time on TN_{plant} ($p = 0.00$). Error bars represent ± SEM.

Figure 5-20 presents the interaction of the soil type and ryegrass cutting time on TN_{plant}. Apart from the third ryegrass cut (02.11.2011), a general declining trend with time was observed. Despite a decline of TN_{plant} for the second cut, TN_{plant} increased significantly for both soil types for the third cut. According to **Figure 5-20**, TN_{plant} in the sandy loam were 2.35, 1.51, 2.10 and 1.63% for the first to last cut respectively while in the clay loam it was 3.15, 2.24, 2.46 and 1.46% respectively for the first to last cut. As mentioned above, the combinations of compost and STSE did not significantly influence TN_{plant}, but a *post-hoc* analysis in Statistica showed that TN_{plant} was significantly higher for the treatment with STSE alone (($0_{compost}+100_{effluent}$)) as compared to the rest of the treatment combinations. Mean TN_{plant} were 2.25, 2.07, 2.03, 2.11 and 2.11% for ($0_{compost}+100_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) treatments respectively.

Figure 5-21 shows the relationship between ryegrass DM yield and the concentration of N in plant materials for the sandy loam and clay loam soils. In the clay loam soil there was no correlation between ryegrass DM yield and TN_{plant} in the lysimeter experiment while the correlation coefficient in the sandy loam was 59%. However, as mentioned in

Chapter 4, in most combinations of compost and STSE, an increase in TN_{plant} did not translate into a higher DM yield. With TN_{plant} of 2.01%, total DM yield was 11672 kg DM ha^{-1} while with TN_{plant} of 1.79%, DM yield was 13179 kg DM ha^{-1} in the sandy loam soil (**Figure 5-21**). Similar observations were made for combined application of compost and STSE in the clay loam soil. The higher losses of N reported earlier in this chapter influenced availability of nutrients in the soil especially in the clay loam soil and subsequently TN_{plant}.

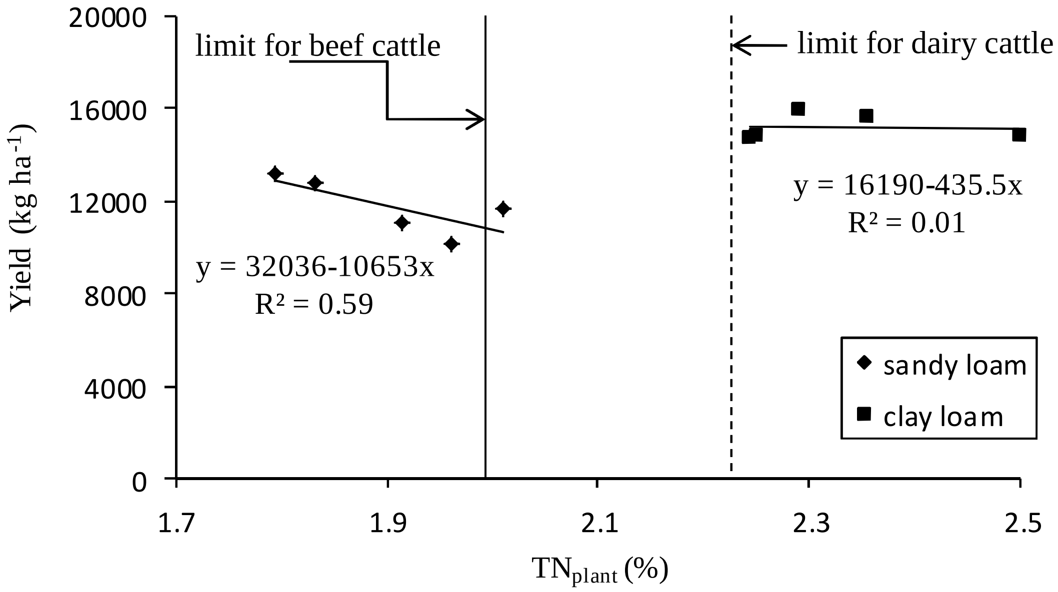
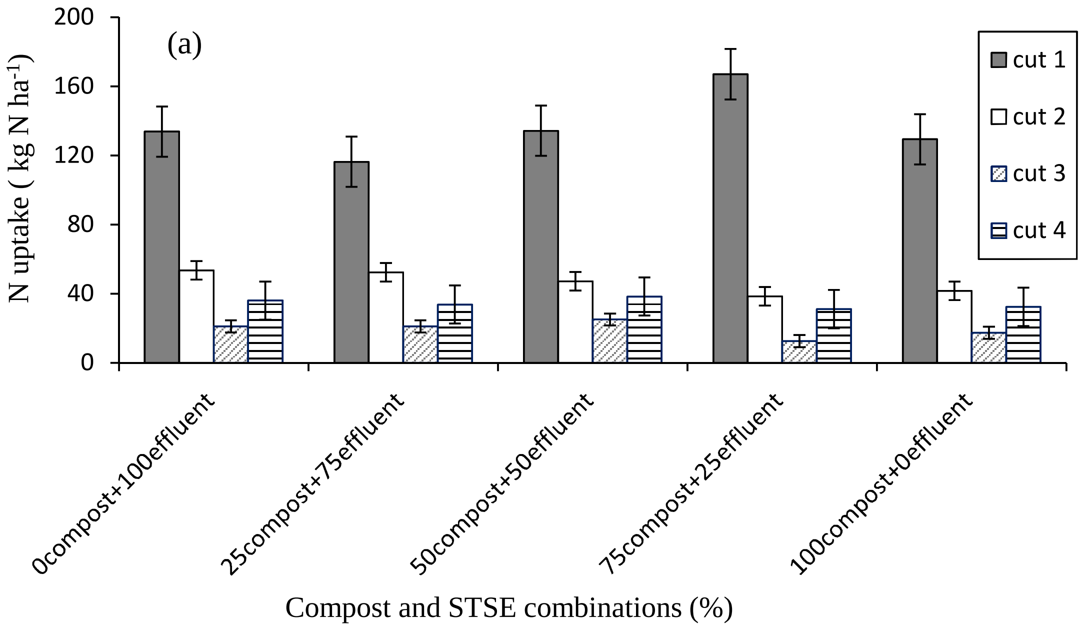


Figure 5-21 Relationship between total ryegrass DM_{yield} and mean TN_{plant} for the lysimeter experiment in sandy loam and clay loam soil.

5.3.4.3 Nitrogen plant uptake

Nitrogen plant uptake (N_{uptake}) was calculated as a product of DM yield and TN_{plant} (Douglas et al., 2003). Since ryegrass was cut at about 2 cm from the ground, N_{uptake} refers only to the above ground DM yield excluding the stumps and the roots. Statistical analysis of N_{uptake} for the four cuts made showed non-significant effect of the combinations of compost and STSE on N_{uptake}.



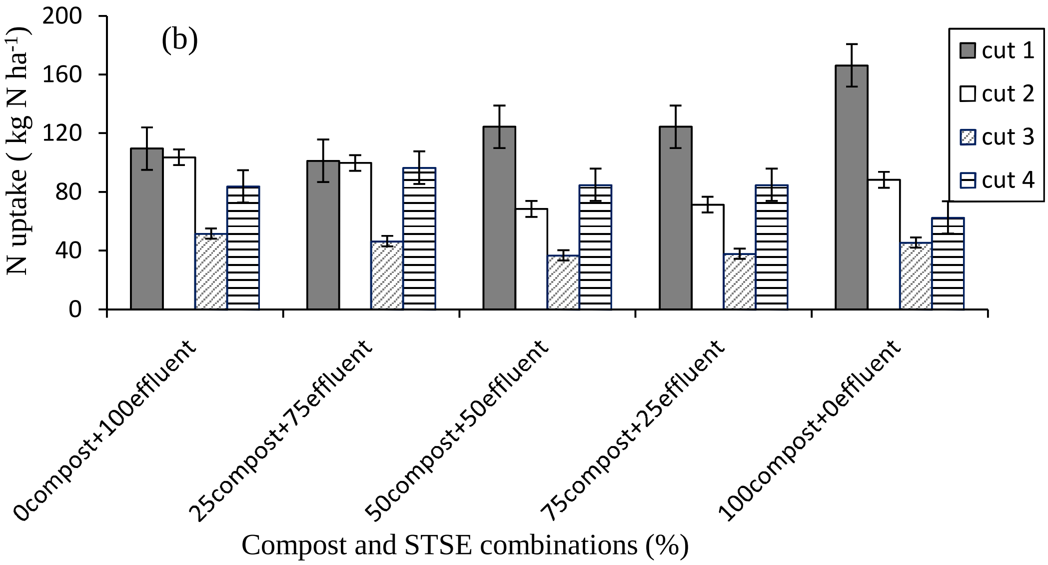


Figure 5-22 N_{uptake} for ryegrass cuts made for the combinations of compost and STSE in a) sandy loam and b) clay loam ($p = 0.13$). Error bars represent ± SEM.

N_{uptake} was significantly influenced by the soil types. Mean N_{uptake} was 59 and 84 kg N ha^{-1} for the sandy loam and the clay loam soils respectively. **Figure 5-22** demonstrates the three way interaction of soil type, ryegrass cuts and combinations of compost and STSE. Though this interaction was not significantly different ($p = 0.13$), N_{uptake} declined significantly with time for the first three cuts. Average N_{uptake} was 131, 66 and 32 kg N ha^{-1} for the first three cuts respectively and 58 kg N ha^{-1} for the last cut on 30 July 2012. **Figure 5-23** shows the interaction of soil type and timing of ryegrass cuts on N_{uptake}. In both soil types, N_{uptake} started to increase after the winter season as witnessed by the increase of N_{uptake} after the third cut.

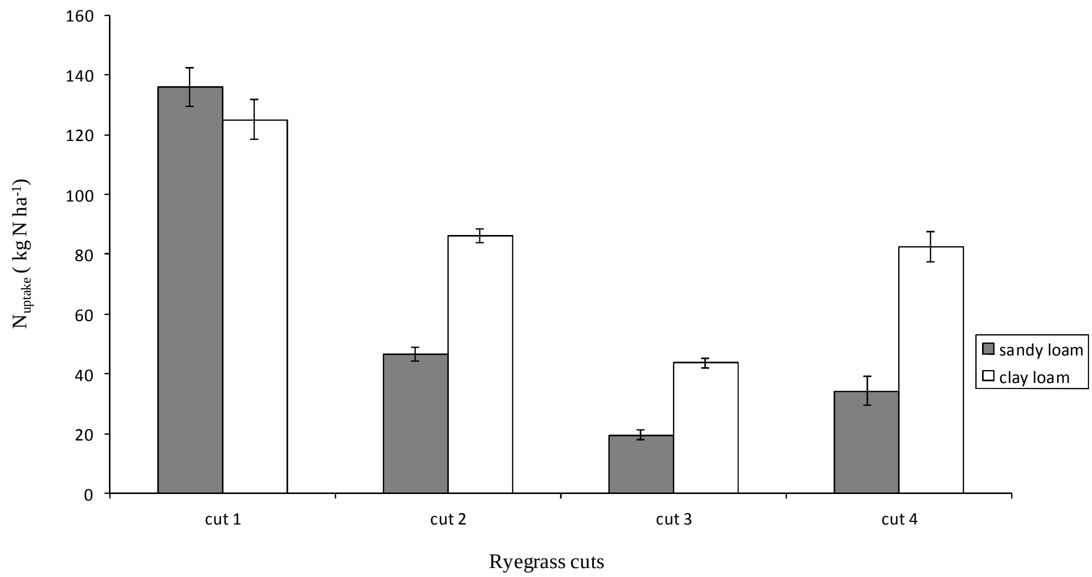


Figure 5-23 Effect of soil type and timing of ryegrass cuts on ryegrass N_{uptake} averaged for the combinations of compost and STSE (P = 0.00). Error bars represent ± SEM.

Factorial ANOVA analysis of total N_{uptake} for the entire duration of the lysimeter (sum of individual cuts for the four ryegrass cuts) showed that the combinations of compost and STSE did not significantly influence total N_{uptake}. Mean total N_{uptake} was significantly higher in the clay loam soil (334 kg N ha^{-1}) as compared to combinations of compost and STSE in the sandy loam soil (237 kg N ha^{-1}).

5.3.4.4 Nitrogen use efficiency

Nitrogen use efficiency (NUE) was estimated using Partial Factor Productivity (PFP_e). PFP_e was calculated using the equation provided in **Chapter 5**. The results of Partial factor productivity for total ryegrass DM yield harvested from the lysimeter experiment have been presented in **Figure 5-24**. Mean NUE for the combinations of compost and STSE was significantly higher ($p < 0.05$) in the sandy loam (90 kg DM kg^{-1} applied N) as compared to the clay loam soil (106 kg DM kg^{-1} applied N). In relation to the combinations of compost and STSE, the interaction of soil type and combinations of compost and STSE (**Figure 5-24**) was significantly different ($p > 0.05$). In both soils,

PFP_e was significantly higher for the treatment ($0_{compost}+100_{effluent}$). Increasing the contribution of compost in combined application of compost and STSE in the sandy loam soil resulted into reduced PFP_e. In the sandy loam, PFP_e was 97, 85, 68 and 74 for the treatments ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) respectively.

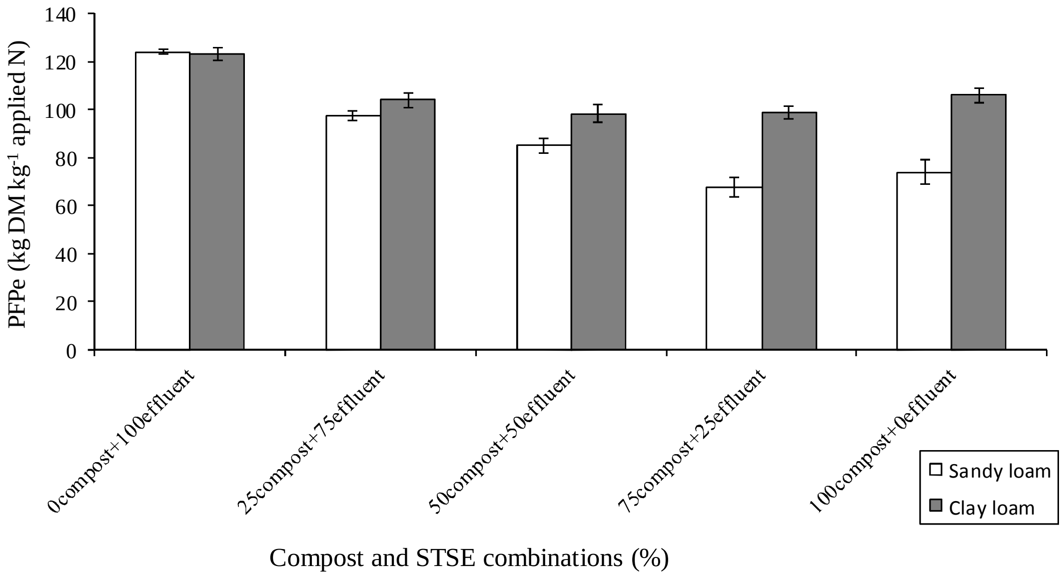


Figure 5-24 Partial factor productivity for total DM harvested in the lysimeter experiment for the combinations of compost and STSE in sandy loam and clay loam soil ($p = 0.00$). Error bars represent ± SEM.

In the clay loam, there were non-significant differences between the treatments ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$). However, mean PFP_e (for the soil types) was significantly higher for treatment with STSE alone, ($0_{compost}+100_{effluent}$) and it declined with increasing compost proportion in combinations of compost and STSE. The results of the influence of the combinations of compost and STSE on PFP_e have been presented in **Figure 5-25**.

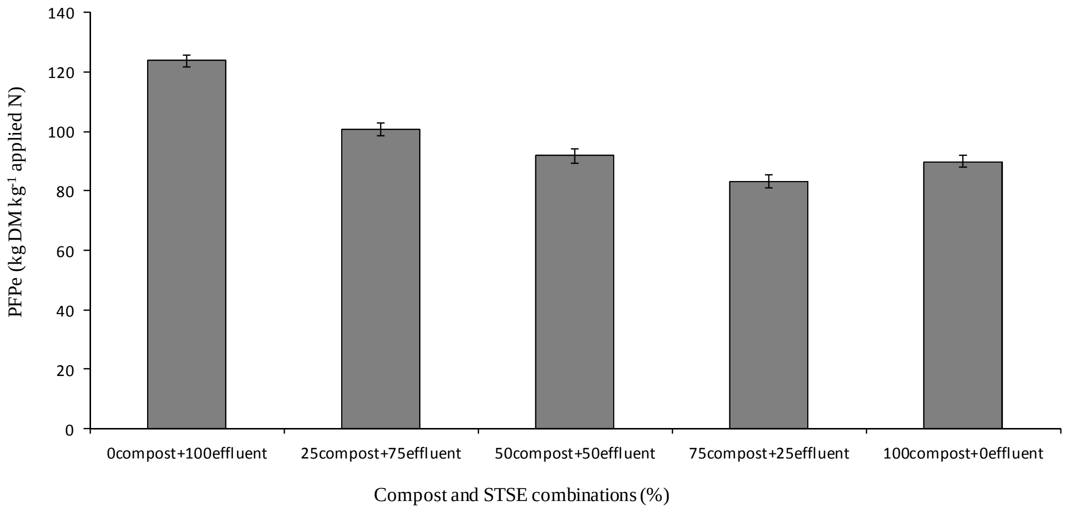


Figure 5-25 Partial factor productivity (mean for the two soil types) for the combinations of compost and STSE (p = 0.00). Error bars represent ± SEM.

5.3.4.5 Phosphorous plant uptake

Phosphorous plant uptake (P_{uptake}) was estimated by considering the DM yield and phosphorous concentration in the ryegrass. P_{uptake} was assessed in all the four ryegrass cuts made during the experiment. As mentioned above, uptake only considered the above ground plant materials from a plant height of about 2 cm above the soil surface.

Repeated measures ANOVA on P_{uptake} for the four cuts showed that P_{uptake} was significantly influenced by the soil type. Mean P_{uptake} per cut for sandy loam and clay loam was 4.19 and 6.48 kg P ha^{-1} respectively. Initial analysis of the soil types before that start of the study showed that total P was higher in the sandy loam as compared with the clay loam but P_{uptake} was significantly higher in the clay loam soil. Since P_{uptake} was determined by considering total P in plant material and ryegrass DM yield, the higher DM yield in the clay loam influenced the calculated P_{uptake}. P_{uptake} was not significantly affected by the combinations of compost and STSE (p = 0.88).

Figure 5-26 presents the interaction of soil type and timing of ryegrass cuts. This interaction was significantly different and it showed a pattern similar to N_{uptake}. P_{uptake} declined from the first to the third cut in both soil types. For the last ryegrass cut (30 July 212), P_{uptake} rose from 1.5 to 3.9 kg P ha^{-1} in sandy loam and 2.6 to 11.1 kg P ha^{-1} in the clay loam soil in comparison to the third cut. Availability of soil P was likely enhanced by the wet period registered between March and July 2012. Weaver et al., (1988) stated that environmental condition (rainfall intensity and temporal distribution of the rainfall) and management practices influence availability of P in soil.

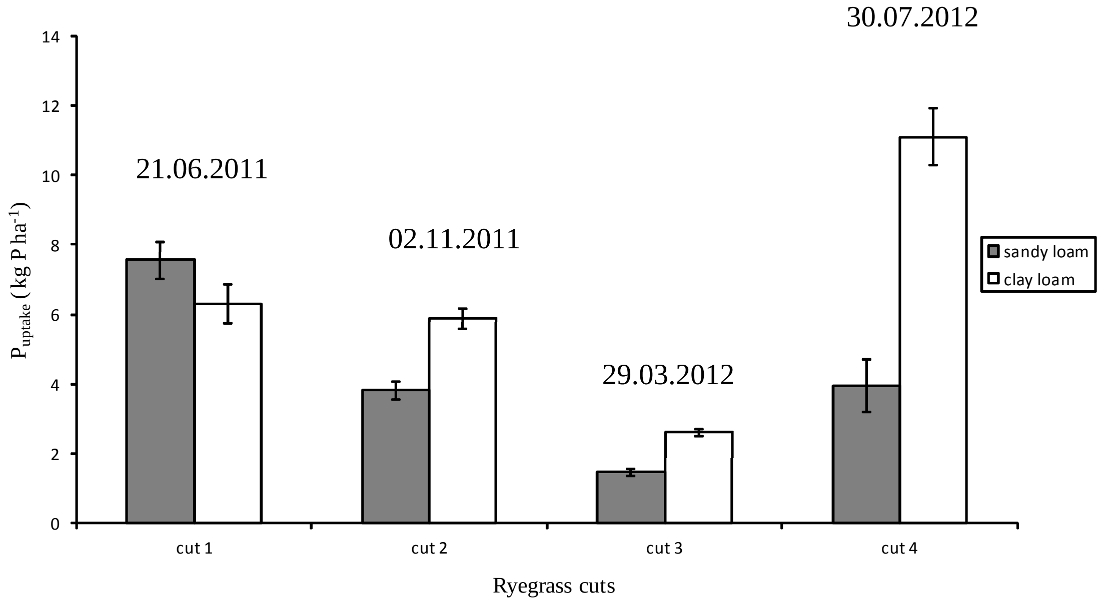


Figure 5-26 The influence of the interaction of soil type and timing of ryegrass cuts on mean P_{uptake} for the combinations of compost and STSE (p = 0.00). Error bars represent ± SEM.

5.3.5 Soil properties

Soil samples were collected during ryegrass cutting/harvesting following the first (21/06/2011) and the last cuts (30/07/2012). Soil samples were collected at two soil depth, 0 to 10 cm and 10 to 50 cm to assess accumulation of plant nutrients and heavy metals in the soil due to the irrigation of STSE on soils amended with greenwaste compost. Statistical analyses were conducted to identify changes that occurred during the study period between the start and at the end of the lysimeter experiment (time effect) for the 0 to 10 cm and 10 to 50 cm soil depth and within the soil profile at the end (to assess build and accumulation of nutrients within the soil profile) in both soil types. Comparisons were also made for soil samples collected at the end of the study (last ryegrass cut) to identify changes in soil chemical and physical properties within the soil profile.

5.3.5.1 Soil mineral nitrogen

Soil mineral nitrogen (SMN) was determined following the first (21/06/2011), second (02/11/11) and the last ryegrass cut (30/07/12). SMN was not determined following the third ryegrass cut to control the amount of soil taken off from the lysimeters. SMN was determined as the sum of NO_3^--N and NH_4^+-N as nitrite concentration in the soil samples was negligible and undetectable in the *Segmented Flow Analyser*. For soil samples obtained from 0 to 10 cm soil depth, repeated measures ANOVA showed that SMN was significantly different ($p = 0.001$) in relation to soil types only. When averaged for the combinations of compost and STSE, SMN was significantly higher in the clay loam (2.45 mg kg^{-1}) as compared to the sandy loam soil (1.52 mg kg^{-1}). The combinations of compost and STSE did not significantly affect SMN in the top 0 to 10 cm soil depth ($p = 0.31$).

Below 10 cm, SMN was significantly influenced by the combinations of compost and STSE ($p = 0.03$) and soil type ($p = 0.00$). SMN was significantly higher in the clay loam (2.31 mg kg^{-1}) as compared to the sandy loam soil (1.09 mg kg^{-1}) when averaged for the combinations of compost and STSE. SMN encountered for both soils before the start of the experiment were considerably higher; i.e. 18.33 and 24.85 mg kg^{-1} for the clay loam and the sandy loam soils respectively. With time, in the 10 to 50 cm soil depth, SMN significantly declined from 2.74 to 0.94 mg kg^{-1} (for the two soil types and compost-effluent combinations) by the end of the study. **Figure 5-27** shows the non-significant interaction of soil type and combinations of compost and STSE. In the clay loam, the treatment ($25_{compost}$+$75_{effluent}$) had the highest SMN of 3.58 mg kg^{-1} as compared to the rest of the combinations of compost and STSE in the clay loam. In the sandy loam, SMN was not significantly different between all the combinations of compost and STSE.

Analysis of soil samples for SMN within the soil profile at the end of the lysimeter study indicated that soil type and combinations of compost and STSE did not significantly effluence SMN. SMN was significantly influenced by the two soil depths from which soil samples were obtained. Averaged for the soil types and combinations of compost and STSE, SMN declined from 1.23 to 0.94 mg kg^{-1} within the soil profile. Nevertheless, the levels of SMN for all the combinations of compost and STSE were low due to ryegrass uptake of SMN. SMN declined significantly ($p < 0.05$) in all the combinations of compost and STSE with time.

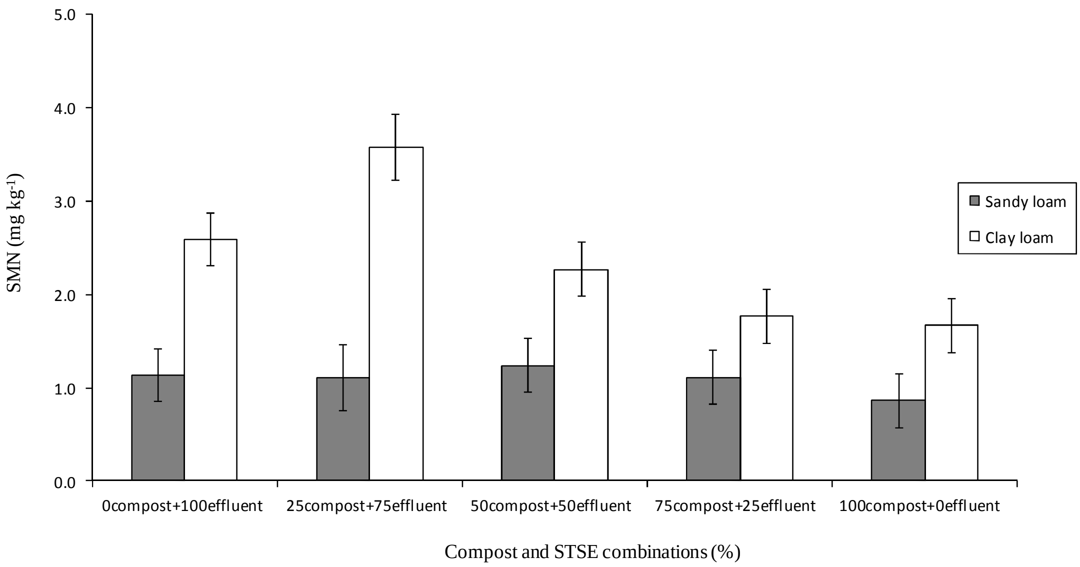


Figure 5-27 The influence of the interaction of soil type and combinations of compost and STSE in the clay loam and sandy loam soils (p = 0.08). Error bars represent ± SEM.

5.3.5.2 Soil total nitrogen

Statistical analysis of soil total N (TN_{soil}) for 0 to 10 cm soil depth for the first and last cuts indicated that there were significant differences between soil types and combinations of compost and STSE. TN_{soil} was significantly higher in the clay loam soil (0.21%) as compared to the sandy loam soil (0.16%). TN_{soil} for treatment ($0_{compost}$+$100_{effluent}$) was significantly lower as compared to all the other treatments. An increase in TN_{soil} was related to an increase in the contribution of compost in combinations of compost and STSE. Within the 10 to 50 cm soil depth, the combinations of compost and STSE did not influence TN_{soil} but TN_{soil} was significantly influenced by soil type, time and the interaction of soil type and time. TN_{soil} increased significantly with time within the 10 to 50 cm soil depth and was significantly higher in the clay loam as compared to the sandy loam soil (**Figure 5-28**).

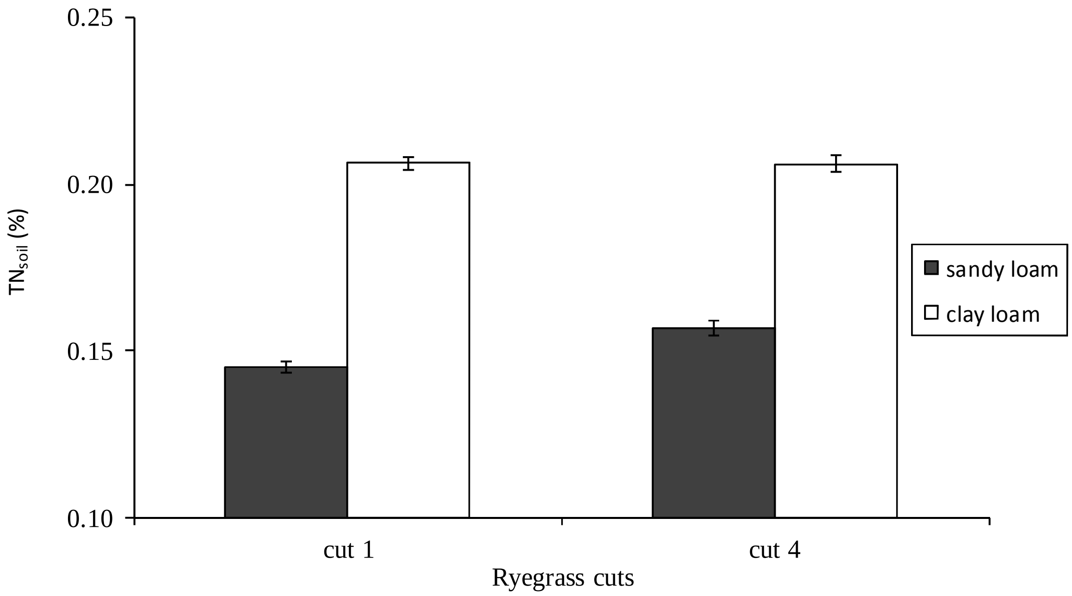


Figure 5-28 Mean TN_{soil} for soil samples sampled within 10 to 50 cm soil depth for the first and last cut in the sandy loam and clay loam soils (p = 0.00). Error bars represent ± SEM.

Assessing TN_{soil} within the soil profile for the last cut showed that TN_{soil} was influenced by soil type and soil depth. Since TN_{soil} was significantly lower (p = 0.013) within 10 to 50 cm soil depth (0.18%) as compared to 0 to 10 cm (0.19%), TN_{soil} did not accumulate/increase within the soil profile in the soil during the study period. In relation to soil type, TN_{soil} was significantly higher in the clay loam (0.21%) as compared to the sandy loam (0.16%).

5.3.5.3 Soil total carbon

Total soil C was significantly influenced by soil types and the combinations of compost and STSE in the 0 to 10 cm soil depth during the study period. In the sandy loam, soil C was 1.7% (w w^{-1}) as compared to *c.* 3.0% (w w^{-1}) in the clay loam. Mean soil C was significantly different between the combinations of compost and STSE. Soil C was significantly lower for ($0_{compost}$+$100_{effluent}$) as compared to the other treatments, but for the other treatments ($25_{compost}$+$75_{effluent}$) ($50_{compost}$+$50_{effluent}$) ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$), soil C was not significantly different. The presence of greenwaste compost influenced soil C in these treatments. The carbon content in compost was 20.7 % (w w^{-1}).

For 10 to 50 cm soil depth, soil C was significantly influenced by the soil type. Soil C declined significantly with time during the duration of the study in the 10 to 50 cm soil depth. However, the combinations of compost and STSE did not significantly influence soil C within this soil depth. Similarly at the end of the study, soil C was not different ($p < 0.05$) within the soil profile but was significantly higher in the clay loam as compared to the sandy loam soil.

5.3.5.4 Soil extractable phosphorous and total soil phosphorous

Analysis of soil extractable P showed that within the 0 to 10 cm soil depth, extractable P significantly accumulated in the soil ($p < 0.05$). After the first cut (averaged for the soil types and combinations of compost and STSE), extractable P was 18.3 mg kg^{-1} while at the end of the study, it was 21.5 mg kg^{-1}. In the lower soil depth range of 10 to 50 cm, extractable P was not significantly different between the first and the last cuts. Extractable P did not accumulate with the soil depth of 10 to 50 cm. However in both soil depths for the first and last cuts, extractable P was significantly higher in the sandy loam as compared to the clay loam soil. Non-significant differences were observed for both depths in relation to the combinations of compost and STSE.

Assessing extractable P within the soil profile for the last cut showed that extractable P was influenced by soil type and soil depth. Soil extractable P was significantly higher in the top 0 to 10 cm soil depth as compared to 10 to 50 cm. There was little downward movement of extractable P within the soil profile in both soils. In relation to soil type, extractable P was significantly higher in the sandy loam (19.5 mg kg^{-1}) as compared to the clay loam (17.6 mg kg^{-1}). Accumulation of extractable P within the soil profile was not influenced by the combinations of compost and STSE.

Analysis of total P showed that with time after comparing the first and last cuts, total P did not accumulate in the 0 to 10 cm soil depth. Total P averaged across the various combinations of compost and treated effluent and soil type was 708 and 520 mg kg^{-1} for the first and the last cuts respectively. Total P was significantly higher with time in the sandy loam (664 mg kg^{-1}) as compared to the clay loam soil (564 mg kg^{-1}) for 0 to 10 cm soil depth. Below 10 cm soil depth, total P did not accumulate in the soil. But like for the 0 to 10 cm soil depth, total P was significantly influenced by the soil type. The interaction of soil type and combinations of compost and STSE was significantly different.

The differences in soil P for both soil depths were as a result of the textural differences of the sandy loam and the clay loam soils used in the study. The results presented in **Section 5.3.1**, showed that the initial concentration of total P was higher in the sandy loam soil than in the clay loam soil while textural analysis showed that the sandy loam and the clay loam had 8 and 31% clay particles respectively ($p < 0.05$). Analysis of cation exchange capacity (CEC) of the soils showed that CEC was significantly higher in the clay loam (17 cmol+ kg^{-1}) as compared to the sandy loam (10 cmol+ kg^{-1}). The higher clay content and CEC would have influenced concentration of total P in the clay loam (Tisdale et al., 1990). But the difference in CEC though statistically different, was small to effectively influence total P in the clay loam.

5.3.5.5 Soil heavy metals

Analysis of heavy metals was done on soil samples from the individual soils before the start of the lysimeter experiment, on soil samples obtained after the first cut (21 June 2011) and following the 4^{th} ryegrass cut on 30 July 2012 which was also the end of the lysimeter experiment. The major purpose of the heavy metal analyses were to establish whether there was build-up of selected heavy metals in the soil following the combined application of compost and STSE or whether STSE accelerated soil heavy accumulation reactions. The heavy metals determined were those of importance due to the well documented effects on the crops, livestock and their potential transfer on to the food chain. Following the first cut, Pb, Ni, Cr, Cu and Zn were analysed from soil samples while at the end of the study only Cu and Zn were analysed as the other heavy metals were too low beyond the detection level of the *AAnalyst 800* Atomic Absorption Spectrophotometer (AAS).

Analysis of Zn showed increasing accumulation within the soil profile. Zn was significantly higher ($P < 0.05$) at the end of the study in the bottom 10 to 50 cm depth as compared to the top soil profile (0 to 10 cm). **Table 5-8** and **Table 5-9** present the results of a three way interaction of soil type, time and the combinations of compost and STSE. With time, the concentration of Zn increased in the 0 to 10 cm soil depth by 15% while in the 10 to 50 cm soil depth, Zn increased by 20%. In both soil depths, the concentration of Zn was significantly higher in the clay loam soil as compared to the sandy loam soil. It is not surprising as initial background conditions also showed that Zn concentration was higher in the clay loam. The combinations of compost and STSE did not significantly influence Zn concentration with time or within the soil profile at the end of the study. Analysis of STSE showed that the concentration of Zn was low and non-detectable but in greenwaste compost, it was 160 mg kg^{-1}. The increase in the concentration of Zn within both the 0 to 10 cm and 10 to 50 cm soil depth was below the maximum permissible concentration in soils reported in **Chapter 4**.

Table 5-8 Concentration of Zinc in the soil following the application of compost and STSE in the sandy loam (p = 0.44 for 0 to 10 cm soil depth and p = 0.47 0 to 10 cm soil depth).

Compost-effluent N combinations (%)	Zinc (mg kg^{-1})			
	Cut 1		Cut 4	
	(0-10) cm	(10-50) cm	(0-10) cm	(10-50) cm
$0_{compost}+100_{effluent}$	44^{a}	43^{a}	33^{a}	48^{a}
$25_{compost}+75_{effluent}$	32^{ac}	47^{a}	47^{a}	53^{a}
$50_{compost}+50_{effluent}$	21^{bc}	44^{a}	51^{a}	57^{a}
$75_{compost}+25_{effluent}$	25^{ac}	49^{a}	33^{a}	51^{a}
$100_{compost}+0_{effluent}$	34^{ac}	43^{a}	40^{a}	48^{a}

Zn values followed by different letters in a column are significantly different ($p < 0.05$).

Soil analysis of Cu showed that at the end of the study, there was no accumulation of Cu within the soil profile. Non-significant effect was found between the two soil depths, 0 to 10 cm and 10 to 50 cm in terms of Cu concentration in the soil. However with time (when averaged for soil type and combinations of compost and STSE), the concentration of Cu declined in the 0 to 10 cm soil depth. Cu declined from 30.9 to 11.7 mg kg^{-1} at the end of the study while in the 10 to 50 cm soil depth, with time Cu declined from 25.7 to 13.1 mg kg^{-1}. The combinations of compost and STSE did not significantly influence Cu concentration with time or within the soil profile at the end of the study.

5.3.5.6 Soil pH

Soil analyses conducted to assess the change in soil pH within the soil profile at the end of the study showed that soil pH was influenced by soil type. Soil pH did not change within the soil profile as soil pH within 0 to 10 cm and 10 to 50 cm soil depths was not statistical different (p = 0.07). The three way interaction of soil depth, soil type and compost-effluent combination was significantly different for the last cut (p = 0.04). The soil pH differences amongst the various combinations of compost and STSE were small.

Table 5-9 Concentration of Zinc in the soil following the application of compost and STSE in clay loam (p = 0.44 for 0 to 10 cm soil depth and p = 0.47 0 to 10 cm soil depth).

Compost-effluent N combinations (%)	Zinc (mg kg^{-1})			
	Cut 1		Cut 4	
	(0-10) cm	(10-50) cm	(0-10) cm	(10-50) cm
$0_{compost}+100_{effluent}$	117[a]	116[a]	131[a]	136[a]
$25_{compost}+75_{effluent}$	113[a]	119[a]	125[a]	156[a]
$50_{compost}+50_{effluent}$	97[a]	114[a]	124[a]	138[a]
$75_{compost}+25_{effluent}$	98[a]	113[a]	119[a]	134[a]
$100_{compost}+0_{effluent}$	114[a]	120[a]	100[a]	146[a]

Zn values followed by different letters in a column are significantly different (p <0.05).

With time, soil pH within 0 to 10 cm soil depth was influenced by soil type, time and the interaction of time and soil type (comparing the first and last cuts). Soil pH increased slightly by 2.6% at the end of the experiment. In the sandy loam (8.04), soil pH was significantly higher than in the clay loam soil (6.96). Compared to soil pH after the first cut below 10 cm soil depth, soil pH was significantly influenced by the combinations of compost and STSE, soil type and interaction of soil type and time. In the sandy loam, soil pH increased from 7.96 to 8.12 while in the clay loam, soil pH increased from 6.74 to 7.18.

5.3.5.7 Soil organic matter

At the end of the lysimeter study, soil organic matter (SOM) was significantly influenced by soil type and the interaction of soil type and combinations of compost and STSE within the soil depth of 0 to 10 cm. SOM was significantly higher in the clay loam (5.73%) as compared to the sandy loam (4.55%). There was a build-up of SOM within the 0 to 10 cm soil depth as mean SOM increased from 4.76 to 5.52% at the end of the study. Compared to initial background condition for the sandy loam and the clay loam soil, mean SOM increased by 17 and 2% respectively.

Similarly, SOM was significantly influenced by soil type, the interaction of time and soil and the three way interaction of soil type, time and compost-STSE combinations within the 10 to 50 cm soil depth. In the 10 to 50 cm soil depth, mean SOM was significantly higher in the clay loam (5.39%) as compared to the sandy loam (4.24%) when averaged for combinations of compost and STSE. However with time, SOM for the 10 to 50 cm soil depth did not significantly change (comparing 10 to 50 cm soil depth at the start and end of the experiment) and the combinations of compost and STSE did not significantly influence SOM.

Analysis of soil samples at the end of the lysimeter study (last cut soil samples) to establish changes of SOM within the soil profile showed that mean SOM (averaged for soil type and combinations of compost and STSE) declined within the soil profile but the combinations of compost and STSE did not significantly influence SOM at the end of the study. SOM declined with soil depth from 5.52 to 4.95% irrespective of soil types and compost-STSE combinations.

5.4 Overall discussion

The results reported for cumulative NO_3^--N leaching showed that the loss of NO_3^--N in the clay loam was higher in treatments ($0_{compost}+100_{effluent}$), ($50_{compost}+50_{effluent}$) and ($75_{compost}+25_{effluent}$). The loss of NO_3^--N through leaching was minimal for the treatment with compost amendment only, ($100_{compost}+0_{effluent}$). Cumulative NO_3^--N loss between the combinations of compost and STSE was significantly different ($p = 0.01$). N losses from organic amendments are mostly driven by volatilisation or leaching in surface waters and groundwater, with only a small amount utilised by the crop or immobilised by the added organic matter (Gregory et al., 2002). NO_3^--N and NH_4^+–N concentrations in soil water may increase as a direct result of inorganic N in the sewage effluent or indirectly through improved soil water status and increased soil organic matter (SOM) mineralisation (Livesley et al., 2007; Myers et al., 1982). An increase of NO_3^--N and

NH_4^+–N in the soil increased its susceptibility to leaching. Whilst cumulative NO_3^--N leaching for the treatment with compost alone, ($100_{compost}+0_{effluent}$) was the lowest at the end of the study (0.54 and 5 kg NO_3^--N ha^{-1} in the sandy loam and the clay loam soils respectively), Basso and Ritchie (2005) found cumulative leaching from compost amended soils of 210 kg NO_3^--N ha^{-1} after a 6 year maize-alfalfa rotation.

Leaching losses of NO_3^--N have been related to uptake of N from the soil by plants (Hepperly et al., 2009) and loss of NO_3^--N can indicate availability of N in the soil. Growing plants often keep NO_3^--N supply depleted to the point that little is lost by leaching (Troeh and Thompson, 2005). With higher N_{uptake}, losses of NO_3^--N in leachate are minimised. Similarly in soils with limited available N, less NO_3^--N becomes susceptible to leaching. In the lysimeter experiment, cumulative NO_3^--N loss was significantly higher in the clay loam soil as compared to the sandy loam soil when averaged for the combinations of compost and STSE. Analyses of TN_{plant}, ryegrass DM yield and N_{uptake} alluded to the fact that the sandy loam soil was less fertile hence less NO_3^--N became susceptible to leaching due to low N mineralisation. In **Chapter 3**, a phenomenon described as “N priming” was mentioned in relation to the higher calculated net N mineralisation in the clay loam especially for the treatments ($0_{compost}+100_{effluent}$) and ($37.5_{compost}+37.5_{effluent}$). N priming in the absence of increased plant N_{uptake} can also contribute to leaching of NO_3^--N. Higher cumulative loss of NO_3^--N in the clay loam as compared to the sandy loam soil was also likely influenced by the fact that the soils were disturbed and the settlement was likely faster in the sandy loam as compared to the clay loam soil.

The concentration of NO_3^--N in leachate is a significant measure for assessing suitability of water for drinking purpose. The concentration of NO_3^--N in leachate during the lysimeter experiment was significantly influenced by the soil types and combinations of compost and STSE. Mean NO_3^--N concentration in leachate was 11.48 mg l^{-1} and 1.75 mg l^{-1} in the clay loam and the sandy loam soils respectively (for combinations of compost and STSE). Peak NO_3^--N concentration was observed for the treatment ($25_{compost}+75_{effluent}$) in the sandy loam soil. The peaks exceeded or were on the limit for drinking water standard of 10 mg l^{-1} (Thomas et al., 2006). For the rest of the treatments in the sandy loam, the concentration of NO_3^--N was below the drinking water standard of 10 mg l^{-1}.

Assessment of the concentration of NO_3^--N in leachate in the clay loam for the combinations of compost and STSE also showed peaks of NO_3^--N concentration above 10 mg l^{-1} largely in treatments with effluent contribution. The highest concentration of NO_3^--N was for the treatment ($0_{compost}$+$100_{effluent}$). The highest concentration of NO_3^--N of 59 mg l^{-1} was observed for the treatments ($0_{compost}$+$100_{effluent}$). Addition of STSE can increase the risk of drinking water pollution in shallow aquifers thereby increasing the risk of methemoglobinemia in infants and young animals. Using sprinkler irrigation system and supplying secondary STSE at normal rates, Livesley et al., (2007) found NO_3^--N concentration to be less than 10 mg l^{-1} with the concentration of NO_3^--N increasing with increasing irrigation application rates. The concentration of NO_3^--N in leachate is likely to be above 10 mg l^{-1} in treatments with STSE alone due to higher effluent application rates.

The results obtained from the analysis of TDN showed that when averaged for all the combinations of compost and STSE and sampling times, loss of TDN was significantly higher ($p < 0.05$) in the clay loam (10.86 kg ha^{-1}) as compared with the sandy loam (0.88 kg ha^{-1}) soil. Cumulatively, TDN loss was higher in treatments with effluent alone or effluent contribution. The highest cumulative TDN loss was in treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$).

Occurrence of sidewall flow has been a major challenge to the use of lysimeters for studying the mobility of contaminants and evaluations of solute transport models. Preferential flow is an artificial channelling of water due to occurrence of air space between the test material and the inside wall of the lysimeter (Hansen et al., 2000; Larsson et al., 1999). A larger proportion of TDN leached was in the form of NO_3^--N. As the analysis of NH_4^+–N showed low concentration of NH_4^+–N in leachate, the remaining part of the TDN was organic N. NH_4^+–N nitrification to NO_3^--N likely resulted in reduced levels of NH_4^+–N in leachate. Preferential flow increases with surface ponding of water common under flood irrigation (Livesley et al., 2007). It is also of concern in lysimeter studies with disturbed/repacked soil. Preferential flow can result in over estimation of leaching rates and under estimation of nutrient leaching as the leachate does not get in contact with soil. Pakrou and Dillon (2000) showed that deep monolith lysimeters and a long-term study provide better results for estimating drainage and N fluxes to groundwater than shorter term studies and the use of shallow or repacked lysimeters.

Phosphorous (P) losses by leaching and runoff from soil results in inefficient utilisation of fertiliser and increased risk of eutrophication of rivers and estuaries (Weaver et al., 1988). Phosphate concentration in leachate was influenced by the compost-effluent combinations. The highest concentration of PO_4^{3-}-P in leachate was from the treatment ($25_{compost}$+$75_{effluent}$) of 0.12 mg l^{-1}. Susceptibility of PO_4^{3-}-P to leach is sometimes controlled by the possibility of P adsorption onto soil particles or up taken by plant roots. Much as non-significant soil type effect was observed for PO_4^{3-}-P leaching, sandy soils are especially prone to P leaching as these soils typically contain less adsorption sites than heavier soils (Eghball, 2003).

Ryegrass DM yields varied within the study period, amongst the combinations of compost and STSE and soil types. Ryegrass DM yield was significantly influenced by soil types. Due to the distinct nature of the soils used, DM yield was significantly higher in the clay loam soil as compared to the sandy loam soil. Averaged between the ryegrass cuts and the compost-effluent combinations, ryegrass DM yield increased in the clay loam by 29%. Clay loam was rich in organic matter; with *c.* 5.7% as compared to *c.* 4% OM in the sandy loam and a C/N of 11 and *c.*15 for the clay loam and the sandy loam soils respectively.

Ryegrass DM yield was significantly higher for treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) as compared to the rest of the compost-effluent combinations (repeated measures ANOVA for all the five cuts). Mean DM yield per cut was 3596 and 3441 kg DM ha^{-1} for ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) treatments respectively (average for the four cuts and soil type). In relation to NO_3^--N leaching, the treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) registered higher NO_3^--N losses yet DM yield was also higher. NO_3^--N loss through leaching did not influenced ryegrass DM yield for the treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$).

The treatment with effluent alone, ($0_{compost}$+$100_{effluent}$) despite providing nutrients in readily available form, it showed low average ryegrass DM yields (average for the two soils). This is contrary to the results that were obtained for the pot/glasshouse study (**Chapter 4**). But as explained earlier on, total N supplied for the treatment ($0_{compost}$+$100_{effluent}$) was below the planned amount of 150 kg N ha^{-1} due to the extreme wet weather experienced in the spring and summer of 2012 affecting crop evapotranspiration and effluent irrigation. But analysis of total DM yield (summation of DM yield for all the four cuts), showed that in the clay loam, the combinations of compost and STSE did not significantly influence total DM. However total DM was significantly higher for the treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) when averaged for the two soil types. However, analysis of NUE using partial factor productivity (that considers the amount of total N applied) showed that in both soils types, NUE was significantly higher for the treatment with STSE alone ($0_{compost}$+$100_{effluent}$). This was in agreement with observation made in **Chapter 4**. In all the combinations of compost and treated sewage, ryegrass DM yield was also likely influenced by the environment. Morrison et al., (1980) concluded in a field study that the seasonal distribution of ryegrass dry matter yield is modified by weather, N supply and soil.

A *post-hoc* statistical analysis in Statistica showed that mean TN_{plant} (for the two soil types) was significantly higher for the treatment with STSE alone, ($0_{compost}$+$100_{effluent}$) as compared to the rest of the treatment combinations. TN_{plant} was significantly influenced by the soil types. As discussed in **Chapter 4**, Wilkins et al., (2000) indicated that for productive grazing animals the minimum level of N in herbage required is 20 g N kg^{-1} while for higher producing milk dairy cows, the range required for N herbage is 2.2 – 2.7% (Aavola and Karner, 2008). The mean TN_{plant} concentration for the treatments ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$), ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) was 2.25, 2.07, 2.03, 2.11 and 2.11% respectively that was above the minimum requirement for N in herbage for productive grazing animals.

Assessment of soil properties after the first cut and at the end of the study for TN_{soil} showed that soil type and compost-effluent nutrient combination significantly influenced TN_{soil}. Overall, TN_{soil} was significantly higher in the clay loam as compared to the sandy loam soil. Compared to the background TN_{soil} of the soils before the lysimeter study, in the sandy loam TN_{soil} declined from 0.2% to 0.16% while in the clay loam soil, TN_{soil} increased from 0.14 to 0.21%. Increasing the contribution of compost in a combination of compost and STSE, increased TN_{soil}. The response of the treatment with effluent alone, ($0_{compost}$+$100_{effluent}$) did not agree with the findings of Fonseca et al., (2007b) who concluded that TN_{soil} concentration increased in the soil following application of secondary STSE in an incubation experiment. Similarly, Quinn and Woods (1978) and Friedel et al., (2000) in longer term field experiments reported an increase in TN_{soil}. At the end of the study, the combinations of compost and STSE did not significantly influence TN_{soil}. But when averaged for the soil types and compost-effluent nutrient combinations, TN_{soil} decreased below 10 cm soil depth.

Soil organic matter (OM) analysed at the end of the lysimeter study showed that integration of compost and STSE will likely increase soil organic matter. Compared to the background values of SOM in the clay loam (5.65%), SOM increased to 6.38, 6.20, 6.04, 6.14 and 6.19% for the treatment ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively. Similarly in sandy soil, soil OM increased from 3.89% (background value in the sandy loam) to 4.16, 4.22, 4.28, 4.43 and 4.34% for the treatments ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$). However, in short term the actual differences between the various combinations of compost and STSE may not be significantly different from each other. This observation is in agreement with an observation made in **Chapter 4**. Application of compost has been associated with increased levels of soil OM. Increased OM in treatments receiving STSE and compost promotes improved soil structure and microbial proliferation (Hargreaves et al., 2008; Gupta et al., 1998; Xu et al., 2012).

Analyses of soil extractable P indicated that with time, there was accumulation of extractable P in the top 0 to 10 cm soil depth. With time (following the first ryegrass cut), extractable P increased from 18.3 to 21.5 mg kg^{-1} (average for soil types and combinations of compost and STSE). Below 10 cm soil depth, extractable P did not change with time. Soil textural differences influenced the concentration of soil extractable P in the soil. Soil extractable P was significantly higher in the sandy loam as compared to the clay loam soil. Similar observation was made for total P. Both total P and extractable P were not influenced by the combinations of compost and STSE. Soil extractable P declined with soil depth at the end of the lysimeter study. It was not surprising therefore that the loss of P through leaching was also minimal as extractable P did not accumulate within the soil profile.

The changes in soil pH between 0 to 10 cm and 10 to 50 cm soil depth at the end of the study were not significant despite the overall increase in the values recorded between the start and the end of the experiment; i.e. from 7.68 to 8.12 in the sandy loam soil and from 6.85 to 7.18 in the clay loam. There is evidence that soil pH has little or no direct effect on plant growth so long as other factors remain favourable (Troeh and Thompson, 2005). Increase in soil pH observed in the two soils could be due to deposition of Ca^{2+} and Mg^{2+} from irrigation with STSE. However, the combinations of compost and STSE had no significant influence on soil pH within the 0 to 10 cm soil depth.

Heavy metals analysis focused on the accumulation of Cu, Zn, Cr, Pb and Ni in the soil due to addition of the combinations of compost and STSE. These elements are generally of concern when compost or effluent is applied to provide water or plant nutrients (Smith, 2009; Lottermoser, 2012) and have documented effects on the crops, livestock and their potential transfer on to the food chain (MAFF, 1998; Dougherty, 1999). The main focus of heavy metals at the end of the lysimeter study was on Zn and Cu as the concentration of Cr, Pb and Ni at the end of the study was non-detectable within the soil profile.

Zn accumulated within the soil profile by the end of the experiment. The mean concentration of Zn within the 0 to 10 cm soil depth at the end of the study was 80.11 mg kg^{-1} while for the 10 to 50 cm soil depth (for the combinations of compost and STSE and soil types), it was 96.68 mg kg^{-1}. In relation to the soil type, Zn was higher in the clay loam (130.75 mg kg^{-1}) as compared to the sandy loam (46.05 mg kg^{-1}), representing a decline of 6% and 3% in the sandy loam and the clay loam soil respectively as compared to the initial background condition of the soils. Zn was either lost in leachate (Zn concentration in leachate was below the *AAS* detection limit) or was taken up by ryegrass. Düring and Gäth (2002) reported that soil acts as a filter for heavy metals thereby reducing leaching of heavy metals from the soil. But the concentration of Zn was below the maximum permissible concentration of potential toxic elements of 200 mg kg for soil pH of between 6 and 7 (MAFF, 1998).

Soil analysis of Cu showed that at the end of the study, Cu did not accumulate within the soil profile. The combinations of compost and STSE did not influence the concentration of Cu in the soil. However, there was a decline in the concentration of Cu when averaged for the compost-effluent combinations and soil type in the 0 to 10 cm soil depth (from 30.9 to 11.7 mg kg^{-1}). Similarly in the 10 to 50 cm soil depth, Cu declined significantly. Using treated wastewater on tomato plants, Samaras et al., (2009) reported no effect on Cu concentration in the soil.

Increase in the concentration of heavy metals in the soil has been associated with long term irrigation with STSE. Xu et al., (2010) found total concentrations of examined heavy metals (Cu, Zn, Cr, Pb and Ni) in soil profiles, indicating very few changes in the chemical status of soils associated with effluent application of 3 years, but long-term irrigation (more than 8 years) increased soil heavy metal levels and affected their distribution in soil profiles. Similarly after 4 years of effluent irrigation, Smith et al., (1996) reported no change in the concentration of heavy metals. Metal adsorption onto organic matter reduces the availability of total heavy metals in the soils. However in long term, mineralisation of organic matter may release metals into available forms even after cessation of amendments (Walter et al., 2002). Considering the duration of the lysimeter study and the low concentration of heavy metals in the treated effluent reported in **Section 5.3.1,** the heavy metals did not accelerate chemical reactions that could have resulted in enrichment of metal concentration in the soils.

5.5 Conclusion

The main conclusions coming from Chapter 5 are summarised below;

a) Irrespective of soil type, the highest ryegrass DM yield in the study was from treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) as compared to the rest of the combinations of compost and STSE. Average ryegrass DM yield for the ryegrass cuts was 3596 and 3441 kg DM ha^{-1} for ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) treatments respectively. Total ryegrass DM yield for the treatment with effluent alone, ($0_{compost}$+$100_{effluent}$) was significantly lower than for treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$). Total ryegrass DM yield was 14583 and 13765 kg ha^{-1} for ($25_{compost}$+$75_{effluent}$) and

($50_{compost}$+$50_{effluent}$) treatments respectively while for ($0_{compost}$+$100_{effluent}$), it was 13237 kg ha^{-1}.

b) N_{uptake} was not affected by the combinations of compost and STSE. N_{uptake} was significantly influenced by soil types. Similarly TN_{plant} was only affected by the soil type and not the combinations of compost and STSE. In the clay loam soil (averaged for the compost-effluent combinations), TN_{plant} increased by 22% as compared to the sandy loam soil.

c) The threat to ground and surface water pollution by NO_3^--N leaching may be enhanced by the inclusion of STSE in integrated compost-effluent nutrient supply to plants. Peak concentration of NO_3^--N of above 10 mg l^{-1} were observed mostly for treatments with effluent N contribution e.g. ($0_{compost}$+$100_{effluent}$) and ($50_{compost}$+$50_{effluent}$).

d) Phosphate concentration in leachate was influenced by the combinations compost and STSE. The highest concentration of PO_4^{3-}-P in leachate was from the treatment ($25_{compost}$+$75_{effluent}$) of 0.12 mg l^{-1} which was above the limit of 0.05 mg l^{-1} but the difference of PO_4^{3-}-P concentration in leachate amongst the various combinations of compost and STSE was small.

e) In the short term, analysis of soil chemical and physical characteristics showed that combinations of compost and STSE did not influence soil physical and chemical properties;

- TN_{soil} within the soil profile at the end of the study was affected by soil type and it decreased with soil depth.
- Soil organic matter increased with time in the 0 to 10cm soil depth and within the soil profile at the end of the lysimeter study, soil organic matter declined with soil depth.
- Soil extractable P and total P accumulated within the soil profile at the end of the lysimeter experiment. In the 0 to 10 cm soil depth with time, soil extractable P increased significantly. In short term, changes in soil properties may likely be influenced by background characteristics of the soil.

f) Soil amendment through combinations of compost and STSE did not influence heavy metal accumulation within the soil profile in the lysimeters. The concentration of Cu and Zn was influenced by soil type and increased with time however; the soil concentration of Cu and Zn was below the maximum permissible concentration of potential toxic elements. Cr, Ni and Pb were undetectable in the lysimeters at the end of the lysimeter study.

6 INTEGRATED DISCUSSION

6.1 Introduction

This chapter discusses the results of the four experiments; two soil incubations, pot/glass house and lysimeter experiments. The results from these experiments have already been reported in **Chapters 3**, **4** and **5**. This chapter provides a concise integration of the results of this research to address the aim and objectives of the research as outlined in **Chapter 1**. Additionally, this chapter outlines the practical considerations for effective implementation and management of compost-effluent integrated nutrient supply. The outline of the research project framework has been provided in **Figure 6-1**.

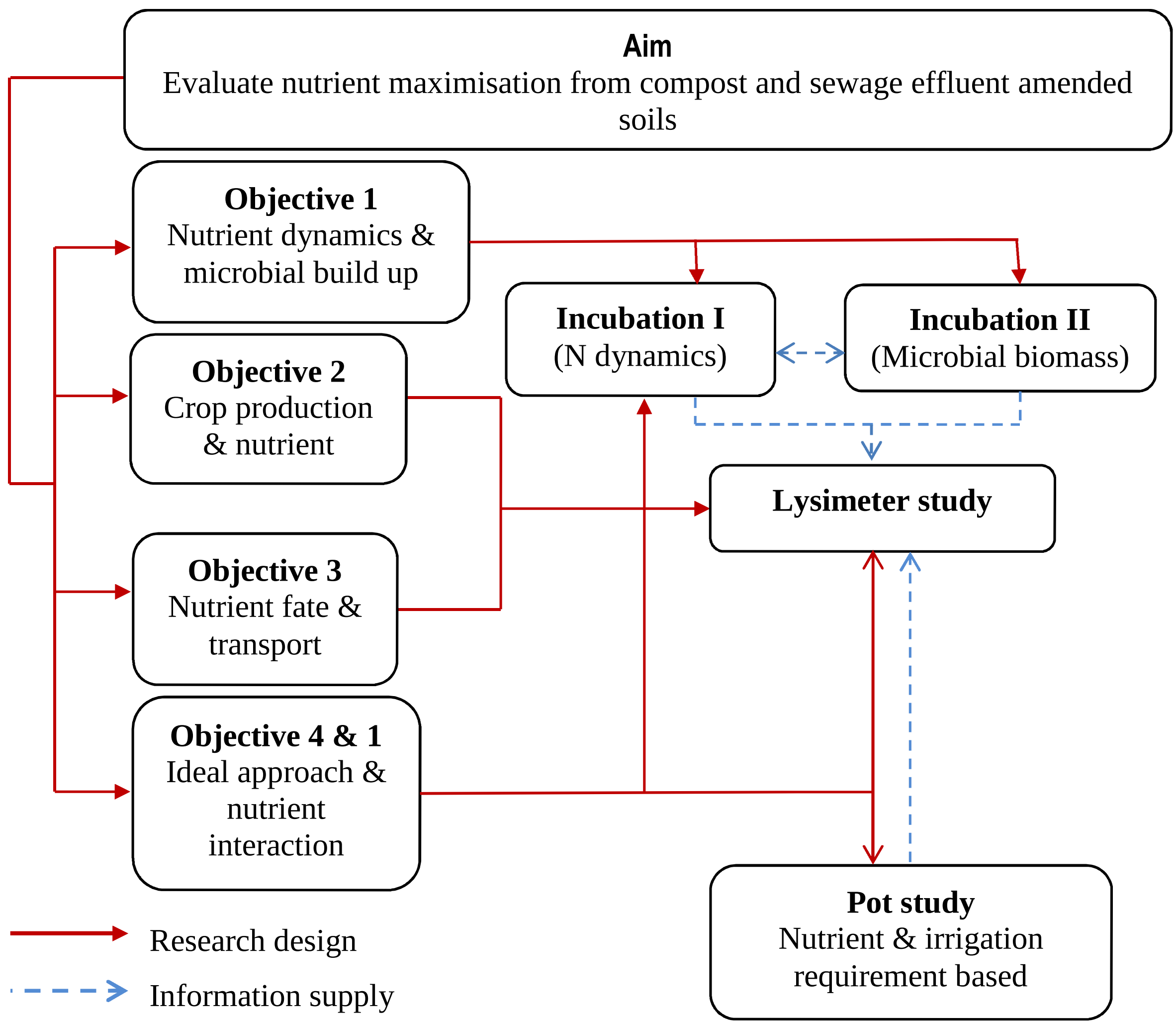


Figure 6-1 Methodological framework for the research project

6.2 Effects on N dynamics

6.2.1 N mineralisation

Nitrogen mineralisation and immobilisation are important processes in the N cycle in soil. Nitrogen mineralisation is the conversion of organic N into ammonium N, whereas N immobilisation is the conversion of inorganic N into organic N (Troeh and Thompson, 2005; Cabrera et al., 2005). Both processes occur simultaneously in soil, with the relative magnitudes determining whether the overall effect is net N mineralisation or net N immobilisation (Cabrera et al., 2005).

Net N mineralisation is controlled by amongst others, organic composition of the organic amendment (Whitmore, 1996), soil temperature and water content (Guntiñas et al., 2012), drying and rewetting events (Kruse et al., 2004) and soil characteristics (Schjønning et al., 1999). Research on N mineralisation has shown that there is a strong relationship between C/N ratios of various types of organic materials and the resulting N mineralisation (Kokkora, 2008; Chadwick et al., 2000; Chaves et al., 2004). For the assimilation of C to occur, N also has to be assimilated in an amount determined by the C to N ratio of the microbial biomass. If the amount of N present in the decomposing organic residue is larger than that required by the microbial biomass, there will be net N mineralisation with release of inorganic N (Cabrera et al., 2005). If the amount of N in the residue is equal to the amount required there will be no net N mineralisation. If, on the other hand, the amount of N present in the residue is smaller than that required by the microbial biomass, additional inorganic N will be immobilised from the soil to complete the decomposition process (Corbeels et al., 1999).

Analysis of initial properties of greenwaste compost used in the study showed that total N in the greenwaste compost was 1.65% which was above the minimum required for mature compost of 0.6% dry weight (Zucconi and De Bertoldi, 1986). Typical values of compost total N in the literature may vary from 0.8% to 3% (Iglesias-Jimenez & Alvarez, 1993; Kokorra: Wolkowski, 2003). As reported by Whitmore (1996), Cabrera et al., (2005) summarised that C/N ratio of residues is related to the amount of N released and that the break-even point between net N mineralisation and N immobilisation can be found between C to N ratios of 20 and 40. With a C/N ratio of 13, greenwaste compost had the potential to release N into the soil assuming nonlimiting environmental conditions. The initial concentration of NH_4^+-N was 476 mg kg^{-1} which was 57% of the total mineral N (NH_4^+-N and NO_3^--N) in the greenwaste compost. The mineral N content of the greenwaste compost was 5% of total N which was above the range of mineral N in compost reported by He et al., (2000) and the quality of the compost was PAS 100 accredited.

A decision to adopt a soil fertility enhancement alternative by farmers amongst others is usually made after considering N availability from amendments. Soils rarely contain enough N for maximum plant growth as witnessed by the pale green colour of N deficiency exhibited by growing plants (Troeh and Thompson, 2005). This was why potential N availability from combinations of compost and STSE was assessed in the incubation experiment.

Overall, NM_{net} was significantly higher in the clay loam soil as compared to the sandy loam soil especially for the treatment ($0_{compost}$ + $37.5_{effluent}$). In the clay loam, mean NM_{net} during the 120 day experiment was 1.6, 0.87, 0.36, 0.41 and 0.26 kg inorganic N kg^{-1} applied N for the ($0_{compost}$ + $37.5_{effluent}$), ($35.7_{compost}$ + $37.5_{effluent}$), ($112.5_{compost}$ + $37.5_{effluent}$), ($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ + $0_{effluent}$) treatment respectively. In the clay loam soil, there was a decline of NM_{net} with increased contribution of compost in combinations of compost and STSE. The higher NM_{net} in treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) could have been influenced by dissolved STSE–N that was largely in mineral form and low C/N ratio of STSE (Fonseca et al., 2007b). According to Livesley et al., (2007), Myers et al., (1982) and Stanford and Epstein (1972), under land-based treatment of effluent, NO_3^--N and NH_4^+-N concentrations in soil water may increase as a direct result of inorganic N in effluent or indirectly through improved soil water status and increased soil organic matter (SOM) mineralisation.

Mineralisation of soil's native organic matter could have also been a source of the extra N mineralisation from the treatments ($0_{compost}$+$37.5_{effluent}$) and ($37.5_{compost}$+$37.5_{effluent}$). According to Diaz et al. (2008), it is possible that some of the N attributed to N sources can originate from the soil organic matter. The readily available N in STSE may have influenced mineralisation of organic matter in the clay loam soil for the treatments ($0_{compost}$+$37.5_{effluent}$) and ($37.5_{compost}$+$37.5_{effluent}$).

Table 6-1 Carbon input and release for the integration of compost and STSE in the sandy loam and the clay loam soils for the incubation experiment.

Compost-effluent combinations (Kg N ha^{-1})	TC_{soil}	MBC	DOC-effluent	*TC_{comp}
	----------	----------	kg ha^{-1}	----------
Sandy loam				
$0_{compost}+37.5_{effluent}$	5844	48	16.8	0
$37.5_{compost}+37.5_{effluent}$	5842	40	16.8	122
$75_{compost}+0_{effluent}$	6045	38	0	242
$112.5_{compost}+37.5_{effluent}$	5630	41	16.8	361
$150_{compost}+0_{effluent}$	6308	42	0	484
Clay loam				
$0_{compost}+37.5_{effluent}$	9013	81	16.8	0
$37.5_{compost}+37.5_{effluent}$	8987	73	16.8	122
$75_{compost}+0_{effluent}$	8799	75	0.00	242
$112.5_{compost}+37.5_{effluent}$	9033	62	16.8	361
$150_{compost}+0_{effluent}$	9100	61	0	484

*TC_{comp} stands for total carbon in compost and TC_{soil} for total carbon in soil.

Addition of STSE in both soils added 16.8 kg ha^{-1} dissolved organic carbon (**Table 6-1**) in the treatments ($0_{compost}+37.5_{effluent}$), ($37.5_{compost}+37.5_{effluent}$) and ($112.5_{compost}+37.5_{effluent}$). Apart from the treatment ($0_{compost}+37.5_{effluent}$), the treatments ($37.5_{compost}+37.5_{effluent}$) and ($112.5_{compost}+37.5_{effluent}$) had extra carbon input from compost. The concentration of C in compost was 21.5% (**Chapter 3**); however the greatest C stock was from the soil's carbon pool (**Table 6-1)**. As discussed in **Chapter 3**, total C was significantly higher in the clay loam as compared to the sandy loam soil ($p < 0.05$). Carbon plays a significant role in microbial metabolism as a source of microbial energy (Brady and Weil, 2008). Marstorp (1996) as reported in Bernal et al., (1998b), in the presence of soluble C, microbial activity is rapidly enhanced such that it can potentially result in degradation of indigenous soil organic matter. The treatments ($0_{compost}+37.5_{effluent}$), ($37.5_{compost}+37.5_{effluent}$) and ($112.5_{compost}+37.5_{effluent}$) had a supply of soluble C that could potentially stimulate higher microbial growth in both soils. However, in the sandy loam higher microbial growth likely resulted in N immobilisation for the treatments ($0_{compost}$+$37.5_{effluent}$), ($37.5_{compost}$+$37.5_{effluent}$) and ($112.5_{compost}$+$37.5_{effluent}$).

In relation to N balance in the soil (incubation experiment), for the treatment with effluent alone, ($0_{compost}$+$37.5_{effluent}$) in the clay loam; based on mean NM_{net} for the 120 days of the incubation experiment reported in **Chapter 3**, 61 kg N ha^{-1} was mineralised (**Table 6-2**). Mineralised N was more than N application rate of 37.5 kg N ha^{-1} that was used for the ($0_{compost}$+$37.5_{effluent}$) treatment. The extra mineralised N was likely from the mineralisation of indigenous organic matter. However, the mechanisms involved in the mineralisation of indigenous organic matter remain unclear (Fontaine et al., 2003; Kuzyakov et al., 2000). In **Table 6-2** losses of ammonia through volatilisation and nitrous oxide were not considered.

Table 6-2 Nitrogen input and release for the integration of compost and STSE in the sandy loam and the clay loam soils for the incubation experiment.

Compost-effluent combinations (Kg N ha^{-1})	TN_{soil}	Effluent-N	Compost-N	N release
	----------	----------kg ha^{-1}	----------	----------
Sandy loam				
$0_{compost}$+$37.5_{effluent}$	494	37.5	0	-13
$37.5_{compost}$+$37.5_{effluent}$	512	37.5	37.5	-20
$75_{compost}$+$0_{effluent}$	515	0	75	-14
$112.5_{compost}$+$37.5_{effluent}$	482	37.5	112.5	-9
$150_{compost}$+$0_{effluent}$	543	0	150	-3
Clay loam				
$0_{compost}$+$37.5_{effluent}$	762	37.5	0	61
$37.5_{compost}$+$37.5_{effluent}$	788	37.5	37.5	65
$75_{compost}$+$0_{effluent}$	747	0	75	31
$112.5_{compost}$+$37.5_{effluent}$	770	37.5	112.5	54
$150_{compost}$+$0_{effluent}$	777	0	150	39

** Negative N release implies nitrogen immobilisation

As presented in **Table 6-2**, 65 and 54 kg N ha^{-1} was mineralised for treatments ($37.5_{compost}$+$37.5_{effluent}$) and ($112.5_{compost}$+$37.5_{effluent}$) respectively. The higher amount of mineralised N in the treatment ($37.5_{compost}$+$37.5_{effluent}$) as compared to the treatment ($112.5_{compost}$+$37.5_{effluent}$) showed the mineralisation efficiency of organic nitrogen.

However, if a comparison is made between the treatments ($37.5_{compost}$+$37.5_{effluent}$) and ($112.5_{compost}$+$37.5_{effluent}$) to ($0_{compost}$+$37.5_{effluent}$), the treatment with effluent alone (($0_{compost}$+$37.5_{effluent}$)) supplied only 37.5 kg N ha^{-1} but mineralised 61 kg N ha^{-1}. Indicating increased N mineralisation efficiency when effluent is applied alone. Saisson et al., (2006) concluded that the response by soil microorganisms is dependent on the application rate of compost, with microorganisms showing resilience when the amount of organic matter is low. However, from this study the response by microorganisms mostly will depend on the quality of the substrate provided.

Increasing compost contribution 3 fold while maintaining the quantity of effluent-N (in the clay loam), e.g. from the treatment ($37.5_{compost}$+$37.5_{effluent}$) to ($112.5_{compost}$+$37.5_{effluent}$) did not result in increased mineralisation of N. N mineralised reduced from 65 to 54 kg N ha^{-1} (**Table 6-2**). From **Table 6-2**, in the absence of compost, there was a possibility of mineralisation of indigenous organic matter in the treatment ($0_{compost}$ + $37.5_{effluent}$). But in the presence of compost (when combined with STSE), the influence of native N (from the soil) was minimal as there was also mineralisation of compost N.

Nitrogen immobilisation is often associated with uptake of nitrogen by microbes; actually other authors have interpreted negative N mineralisation as N immobilisation by microbial biomass (Burgos et al., 2006; Sims, 1990). But, according to Calderón et al., (2005) negative mineralisation does not necessarily relate to N immobilisation by soil microbes because of a possibility of higher denitrification losses. According to Smith et al., (1998), gaseous losses of N by denitrification or volatilisation can potentially cause large errors in estimation of N availability of organic amendments determined by laboratory incubation, depending upon the experimental conditions. In this study negative NM_{net} for combinations of compost and STSE in the sandy loam soil is associated with N immobilisation.

As explained in **Chapter 3**, soil moisture content in the sandy loam and the clay loam soils was maintained at 100% and 98% maximum water holding capacity in the sandy loam and the clay loam soil. The higher moisture content possibly influenced nitrogen loss through nitrous oxide. But soil texture influences the drainage rate of the soil and thus the amount of denitrification loss is often greater in clay soils than in free draining sandy soils (Cameron et al., 2013). However, the higher NM_{net} due to the rewetting and drying cycles likely over shadowed any possible denitrification losses in the clay loam soil.

During the first 30 days in the sandy loam soil (incubation experiment), inorganic N concentration declined rapidly in all the treatments. NM_{net} with time was lower in treatments with higher proportion of compost. Han et al., (2004) observed a decline of inorganic N after integrating urea and compost in soils with different indigenous carbon content. In their study, Han et al., (2004) found increased immobilisation of urea-derived N in soil with low indigenous organic carbon. As reported in **Chapter 3**, organic matter and organic carbon in the sandy loam was lower in comparison to the clay loam soil thereby influencing N mineralisation dynamics. Addition of STSE in the sandy loam likely increased microbial proliferation (added 16.8 kg DOC ha^{-1} - **Table 6-1**) that could not be sustained by the soil's organic carbon and nitrogen resulting in depletion of N (N immobilisation) for the treatments ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$). Studies on N mineralisation from various organic materials (food waste and crop residues) have shown that the decomposability of the carbon sources of materials also influences N mineralisation (Kokkora, 2008; Janssen, 1996).

Input of fresh organic matter triggers activity of dormant microorganisms (De Nobili et al., 2001). As described by Fontaine et al., (2003), microorganisms that specialise in fresh organic matter decomposition (r-strategists) proliferate quickly due to input of readily available source of energy at the expense of slow growing "k-strategist" microorganisms. After substrate exhaustion, the fast growing "r-strategist" microorganisms die off or become dormant as they cannot compete for recalcitrant organic matter. Rapid microbial growth and higher microbial activity in the clay loam soil could have resulted in rapid mineralisation of organic nitrogen. Paré and Gregorich (1999) concluded after amending soil with maize, alfalfa and and soya bean residues that additions of energy-rich materials probably stimulates microbial growth, resulting in a larger and more active microbial biomass thereby speeding the mineralisation of indigenous soil organic matter as well as that of added materials.

In the sandy loam soil, decomposition by the “k-strategist” microorganisms after the exhaustion of the readily available organic matter from STSE and compost was likely slow due to limited availability of indigenous soil organic matter, organic carbon and nitrogen. Nutrient supply in the sandy loam was low to sustain microbial cell synthesis and higher activity levels. In the clay loam, the transition from r-strategist to “k-strategist” microorganisms may not have resulted in N immobilisation as the soil had the potential to supply microbial energy and N for the mineralisation of organic matter.

Soil texture is another important factor that influenced the rate of N mineralisation. Since the loss of N through denitrification is higher in fine textured soils than coarse-textured soil, Parfitt and Salt (2001) concluded that NM_{net} follows the order: sand > clay > silt for soil fractions with 51% sandy, 33% silt and 16% clay. Paré and Gregorich (1999) found lower N mineralisation in soil that had clay content of 54% after amending soil with alfalfa residues. It is assumed that physical protection of organic matter and microbial biomass by clay layers and by aggregate formation affect N mineralisation in fine textured soils hence low N mineralisation. However N mineralisation rates in the incubation experiment suggested otherwise. N mineralisation rates were significantly higher in the clay loam soil ($p < 0.05$) than the sandy loam soils. Soil texture is not always the dominant factor determining the organic C content of soils and C mineralization rates (Hassink, 1994). Actually, on drying and rewetting the protective mechanisms of clay particles are undermined and large amounts of organic N become available for mineralization and subsequent nitrification (Sahrawat, 2008).

In the sandy loam soil, higher NM_{net} was observed at the start of the incubation experiment in treatments with effluent-N contribution, indicating a significant contribution effluent-N made to NM_{net} at the start of the incubation. However, disappearance of the initially higher NH_4^+-N (in the greenwaste compost - 476 mg kg^{-1}) may have also influenced the negative N mineralisation (Calderón et al., 2005). Burgos et al., (2006) also noted markedly reduced NH_4^+ -N until 6 weeks after which NH_4^+ -N content was undetectable. Disappearance of NH_4^+-N has been attributed by Hadas and Portnoy (1994b) and Han et al. (2004) to the assimilation of N by growing microbial biomass stimulated by the organic and inorganic N amendments. According to Fonseca et al. (2007b) loss of NH_4^+ -N is strongly linked to microbial N immobilisation as it is the most preferred microbial inorganic N.

6.2.2 Mechanism of nutrient interaction

Understanding the underlying mechanisms behind N availability due to the interaction of STSE and compost N when applied together, forms the back bone of the integrated compost and STSE nutrient application. Mechanisms of interaction can help to explain the response of the different soil types in relation to N availability due to the integration of compost and treated effluent. It can also provide insights to the differences in N mineralisation for the various combinations of compost and STSE.

Analysis of data from the experiments on soil properties and crop production suggested that the response to the integrated application of compost and STSE was higher in the clay loam as compared to the sandy loam soil. In all the experiments, assessment of initial properties of the clay loam and the sandy loam soils (**Chapter 3 and 5**) showed that the clay loam soil was more fertile as compared to the sandy loam soil.

In the clay loam, treatments with STSE alone or effluent in combination with compost, ($0_{compost}$ + $37.5_{effluent}$), ($37.5_{compost}$ + $37.5_{effluent}$) and ($112.5_{compost}$ + $37.5_{effluent}$) registered higher NM_{net} during the incubation experiment. The release of nitrogen higher than the applied total N has been attributed to the release of initially immobilised N due to death of microbes and also from the microbial cell metabolism (Azeez and Van Averbeke, 2010; Eneji et al., 2002; Abbasi and Khizar, 2012; Mubarak et al., 2010; Khalil et al., 2007). However pre-treatment of soils (drying and rewetting) has also been reported as a possible cause of initial N mineralisation flush (Cabrera et al., 2005). Soil drying can cause changes in soluble organic matter as some of the solubilised organic compounds may come from microbial biomass killed by drying the soil samples (Benbi and Richter, 2002). On rewetting, the dead biomass becomes mineralised rapidly (Kieft et al., 1987; Van Gestel et al., 1991).

Mineralisation of native organic matter could potentially have also been a source of the extra N mineralised for the treatments ($0_{compost}$ + $37.5_{effluent}$) and ($37.5_{compost}$ + $37.5_{effluent}$) in the clay loam. As outlined by Kuzyakoz et al., (2000), the scheme of N priming effect has been presented in **Figure 6-2**. The readily available N and the dissolved organic carbon in STSE may have influenced mineralisation of organic matter in the clay loam soil. Mean dissolved organic C (DOC) in STSE was 88 mg l^{-1} (range 31 to 146 mg l^{-1}). As described by Kuzyakov (2010), the C input to initiate mineralisation of indigenous organic matter can be one time-occasion or continuous.

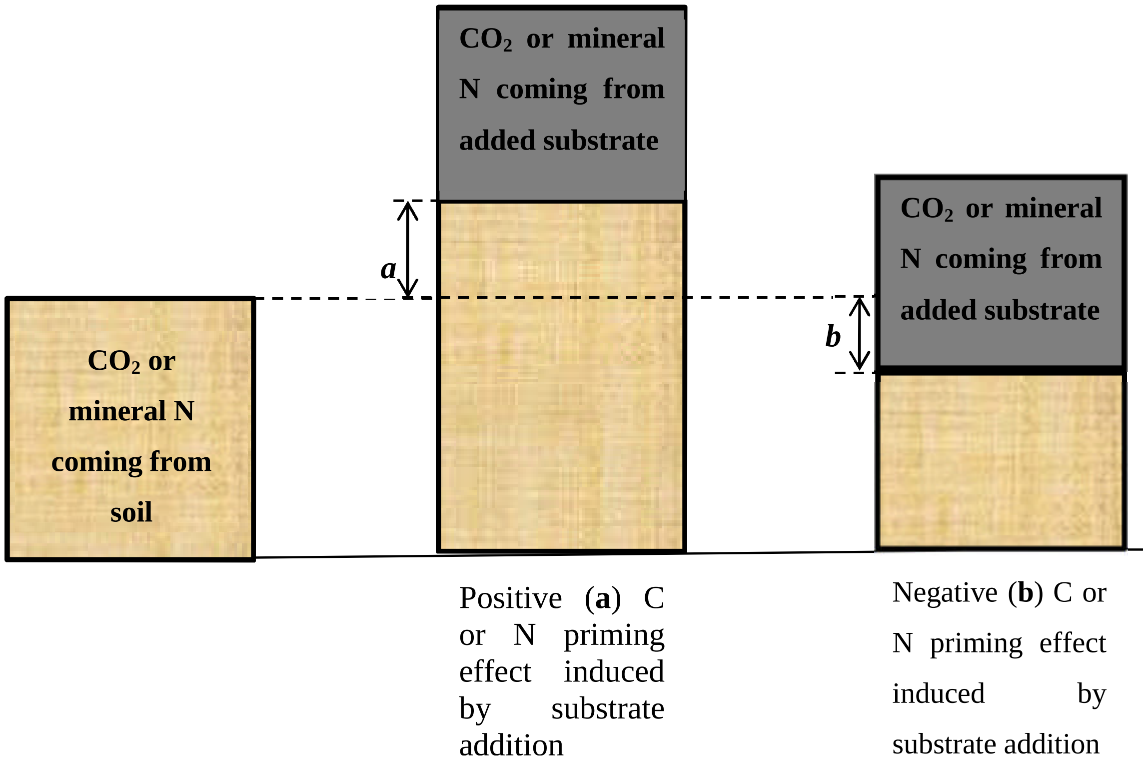


Figure 6-2 Schematisation of the priming effect (a) acceleration of SOM decomposition positive priming effect; (b) retardation of SOM decomposition negative priming effect (Source: Kuzyakov et al., (2000)**).**

Priming effects have been described as being small, short time and occurring immediately or shortly after addition of specific substances to the soil (Fontaine et al., 2003; Kuzyakov et al., 2000). Addition of easily decomposable substrates can result in either positive N priming or can induce negative N priming in the soil. For the incubation experiment, the C input was one off while in the pot and lysimeter experiments, the supply of C through STSE was continuous. The continuous input is typical for the slow decomposition of dead roots, leaf and shoots residues and for some rhizo-deposits (Kuzyakov, 2010). The resultant effects of continuous supply of C (repeated application of STSE) on crop production were presented in **Chapters 4 and 5**.

In these chapters, increasing the contribution of effluent-N did increase ryegrass DM yield and N_{uptake}. However, the underlying mechanisms of N priming remain contentious among various researchers following divergent findings reported in literature (Azam et al., 1993).

It is anticipated that as a result of the priming effect of indegenious soil nitrogen, the concentration of total soil nitrogen will decline with time. Much as priming effect is a possible mechanism for availability of nitrogen in both soils, there was no evidence in terms of soil nitrogen decline at the end of the incubation experiment (**Chapter 3**). In the clay loam, there was no significant decline of soil total N as evidence of the priming effect.

In the sandy loam, with time the extent of N immobilisation reduced with increasing contribution of compost. As discussed earlier on, the low NM_{net} in the sandy loam was related to the initial background condition of the soil before the incubation experiment. The immobilisation that took place in the sandy loam for treatments with combined application of compost and STSE was likely to be the result of microbial proliferation due to the addition of easily decomposable substrates. According to Abbasi and Khizar (2012), the increase in microbial reproductive rates results into a competition for nutrients and subsequent immobilisation of nutrients, largely in the soils with low in fertility. In this research study, the phenomenon was more pronounced in the sandy loam. The extent and the duration of N immobilisation in the sandy loam soil for ($0_{compost} + 37.5_{effluent}$), ($37.5_{compost} + 37.5_{effluent}$) and ($112.5_{compost} + 37.5_{effluent}$) treatments can potentially present serious problems on crop growth due to limited availability of inorganic N.

CEC of the soils (clay loam and sandy loam) is also likely influenced the interaction of nutrients from compost and STSE. In a study by Duong et al, (2012) in soils with different proportions of clay content, they found increased CEC especially in soils with higher clay content (22 and 49%) as a result of compost amendments (garden waste, agricultural residues and manures). Cation exchange capacity (CEC) was 10 and 17 cmol+ kg^{-1} in the sandy loam and the clay loam soils respectively while SOM was 3.7 and 4.8% for the sandy loam and the clay loam soils respectively (**Chapter 3**). The highly decomposed organic matter in compost has a large number of cation binding sites (Duong et al., 2012) that increases the likelihood of NH_4^+-N adsorption. This explains why increasing the contribution of compost in combined application of compost and STSE resulted in reduced NM_{net} in the incubation experiment in the clay loam soil due to NH_4^+-N adsorption.

When the second identical incubation experiment was conducted to study the mechanism of interaction through microbial biomass, inconclusive results were obtained. As outlined in **Chapter 3** (**Section 3.2.4**), in order to supply similar amounts of N through STSE for corresponding treatments in the clay loam and the sandy loam soils, mean of the two field capacities for the clay loam and the sandy loam was used. This ensured similar N application rates for both soil types. However the result was that moisture content was 100 and 98% of water holding capacity for treatments in the sandy loam and the clay loam soils respectively. Moisture condition is a major factor controlling survival and activity of microorganisms in the soil (Zhang et al., 2005). Adequate soil moisture increases microbial biomass. Beyond field capacity, microbial activity decreases with increasing moisture, due to limited oxygen availability. Additionally the methodology used for microbial biomass has limitations related to moisture content of the soil. Chloroform fumigation works in soils with lower moisture content in which case chloroform is uniformly distributed in the soil sample. In soil samples at higher moisture content, microbial biomass may not be exposed to the fumigant leading to underestimation of microbial biomass (Azam et al., 2003).

In summary, multiple mechanisms influenced the dynamics of nitrogen in the interaction of compost and STSE. Cation exchange capacity, quality of available carbon, soil microbial activity and drying and rewetting cycles of soil are the mechanisms involved in nutrient interaction of compost and STSE nutrients. The mechanisms influence release and availability of nutrients and impact of the combinations of compost and STSE on crop production and leaching of nutrients.

6.3 Effects on soil properties

6.3.1 Soil organic matter

Soil organic matter was influenced by soil type and the interaction of soil type and combinations of compost and STSE. SOM was higher in the clay loam as compared to the sandy loam soil. However in both soils, SOM increased at the end of the experiments. For example, in the lysimeter experiment (**Chapter 5**), mean SOM increased by 17 and 2% in the sandy loam and the clay loam respectively while in the pot experiment mean SOM increased by 9% and 4% (from the initial SOM in the soils) in the sandy loam and the clay loam respectively (for the combinations of compost and STSE and application rates).

Assessment of SOM within the soil profile at the end of the lysimeter study showed that SOM was higher in the top 0 to 10 cm soil depth. This was not much of a surprise as the 0 to 10 cm soil depth is full of plant roots and dead plant leaves. In the short term, the study concludes that the integrated application of compost and STSE will influence soil organic matter but the build up will not be different amongst the combinations. Sugiura (2009) reported that SOM build-up in the soil is slow. However, Mohammad and Mazahreh (2003) at higher rate of wastewater application (equivalent to 125% class A pan reading) found that organic matter was higher in the top soil, but not in the subsoil.

6.3.2 Phosphorous

P availability was affected by the combined application of compost and STSE on the sandy loam and the clay loam soils. The effect on soil extractable P was found to be dependent on soil texture. For the lysimeter experiment, irrespective of the sampling depth and combinations of compost and effluent, soil extractable P was also significantly higher in the sandy loam (19.5 mg kg^{-1}) as compared to the clay loam (17.6 mg kg^{-1}). However, the combined application of compost and STSE did not influence soil extractable P in the soil.

In relation to soil extractable P in the soil profile, at the end of the lysimeter experiment the concentration of extractable P was significantly higher in the 0 to 10 cm soil depth as compared to 10 to 50 cm soil depth. Similarly, Suguira (2009) using STSE from the same source (Cranfield Universirty Treatment Plant) reported higher P content in the soil at 10 am soil depth, as compared to 30 and 50 cm soil depth. He et al., (2001) as presented by Diacono and Montemurro (2010) reported an increase in the concentration of P in the 0-15 cm soil depth after a 4-year repeated application of compost. This was an indication that there is little downward movement of extractable P within the soil profile in both soil types. But Kokorra (2008) reported movement of available inorganic

P downwards through the soil profile below the root zone in coarse sandy soil (98% sandy); indicating that movement of P is governed not only by availability of P but also by soil texture. Alshammary and Qian (2008) concluded after long term effluent irrigation on golf courses (35 years) that P levels in the upper soil structure were very higher and advocated proper management of waste water irrigation and monitoring to ensure successful, safe and long term waste water irrigation. Similarly, Yadav et al., (2002) reported that surface soil was richer in P than the underlying layers at all sites where effluent irrigation had been taking place for about 30 years.

6.3.3 Soil nitrogen

The study has shown that nutrient combination of compost and STSE can influence TN_{soil} especially in the top soil profile (0 to 10 cm soil depth). In the 0 to 10 cm soil depth, an increase in TN_{soil} was related to increasing contribution of compost in combinations of compost and STSE. This observation did not agree with conclusions made by Fonseca at al., (2007) that TN_{soil} increased with STSE application rates. Compost application is likely to increase TN_{soil} especially at higher N application rate (Kokkora, 2008). In a 4-year study where different organic amendments were applied to water melons, Lopedota et al., (2013) found no significant change in soil nitrogen. In the research study, no differences in TN_{soil} were noted between the various combinations of compost and STSE.

TN_{soil} reduced with depth in the soil profile (0.18 and 0.19% (w w^{-1}) for 10 to 50 cm and 0 to 10 cm soil depth respectively). However the actual difference between the two soil depths was too low to make any significant difference in practice. The combinations of compost and STSE did not significantly affect TN_{soil}.

6.3.4 Heavy metals

The major purpose of the heavy metal analyses were to establish whether there was build-up of selected heavy metals in the soil following the combined application of compost and STSE or whether STSE accelerated soil heavy metal accumulation reactions. The analyses of selected heavy metals (Cr, Ni, Cu, Zn, and Pb) did not show significant changes ($p > 0.05$) in their levels as compared with those recorded at the start of the experiments. In the pot experiment, significant soil type effect was noted for

Cu, Pb, Cr, and Zn. When averaged for all the combinations of compost and STSE and soil type; Cu, Pb and Ni concentration increased by the end of the pot experiment. However, the values reported were found to be well below the maximum permissible/advisable concentrations given the soil pH values encountered in the experiments (Cela and Sumner, 2002).

For all the heavy metals analysed during the research study, the combinations of compost and STSE did not significantly influence heavy metal concentration with time or within the soil profile by the end of the study. Combined application of compost and STSE in short term is likely not to present any risk of heavy metal accumulation above the maximum permissible concentration in soil if effluent of similar characteristics is used. It is obvious that the duration of the research study was not long enough to establish future trends of heavy metals dynamics in the soil. However, following the short term trends established for some heavy metals, e.g. Zn, close monitoring of the soil can be essential to ascertain that the build-up of metals is not above the maximum permissible heavy metal limits.

Overall the dynamics of heavy metals in the lysimeter experiment agreed with observations made in pot experiment. The quality of the STSE used in the study influenced the dynamics of the heavy metals in the soil. STSE from sewage of household origin has low concentration of heavy metals as compared to that from industrial areas (Al-Musharafi et al., 2012). But also once STSE contain less proportion of heavy metals as most of the heavy metals end up in sludge (Emongor and Ramolemana, 2004). However Gwenzi and Munondo (2008) found that application of STSE over the years (26 years) has led to significant increases in some total heavy metal concentrations.

6.4 Crop production

6.4.1 Ryegrass dry matter

Analysis of annual ryegrass DM yield for the first year in the glasshouse study has shown that DM yield was influenced by the combination of compost and STSE, soil type and N application rates. In the first year (2010/11) in the sandy loam soil, higher DM yield was harvested from the treatment ($0_{compost}+100_{effluent}$) at N application rate of

150 kg total N ha^{-1} (7321 kg DM ha^{-1}). When compost was added to the combinations of compost and STSE as with the treatment ($25_{compost}+75_{effluent}$), DM yield reduced to 6008 kg DM ha^{-1}. Ryegrass DM reduced further for the treatment ($50_{compost}+50_{effluent}$) to 4680 kg DM ha^{-1}. A similar trend was observed in the same year (2010/11) but at lower N application rate of 75 kg N ha^{-1} in the sandy loam.

In **Chapter 3**, it was concluded that increasing the quantity of compost in compost and STSE nutrient integration resulted in reduced net N mineralisation. Hence net N mineralisation was higher in treatments with effluent alone in the clay loam soil. The higher net N mineralisation in treatments with less compost contribution reported in **Chapter 3** corresponded with the trends established for ryegrass DM yield discussed above. DM yield was similarly higher in similar treatments that had higher net N mineralisation in the incubation experiment.

Similarly in the clay loam soil, ryegrass DM yield in the first year (2010/11) for the pot experiment was higher ($p < 0.05$) for treatments with STSE alone ($0_{compost}+100_{effluent}$). Ryegrass DM yield decreased with increasing compost contribution. In the second year, ryegrass DM was not significantly different ($p > 0.05$) between the treatment with effluent alone ($0_{compost}+100_{effluent}$) and the treatment with minimal compost contribution, ($25_{compost}+75_{effluent}$). The effect of repeated and continuous application of compost and STSE was apparent for these two treatments in the clay loam. Although the same pattern was not replicated in the sandy loam, it showed the potential that the treatment with less compost contribution (($25_{compost}+75_{effluent}$)) can have as compared to treatment with STSE alone, ($0_{compost}+100_{effluent}$) in the long term. This pattern agreed with observations made by Sikora and Enkiri (1999) that a combination containing one-third biosolids compost-N and two-thirds fertiliser-N resulted in fescue yield not significantly different from 100% fertiliser. The synergetic effect of compost on dissolved inorganic N in STSE resulted in crop benefit from the addition of organic matter and readily available N from compost and effluent respectively.

Table 6-3 summarises the results of mean total ryegrass DM yield in the pot experiment. The results of ryegrass DM yield shows that it is not necessary to increase STSE contribution e.g. from the treatment ($25_{compost}+75_{effluent}$) to ($0_{compost}+100_{effluent}$) as the yield difference is barely significant. Instead, increase in STSE will increase susceptibility of readily available nutrients to leaching.

Table 6-3 Mean total ryegrass DM yield (kg ha^{-1}) averaged for the two years of the pot experiment for the sandy loam and the clay loam soils at the two N application rates of 75 and 150 kg N ha^{-1} (p =0.038). Numbers in a column with different letters are significantly different.

Compost-effluent combinations (%)	Sandy loam		Clay loam	
	N application rates (kg N ha^{-1})			
	75	150	75	150
$0_{compost}$ + $100_{effluent}$	5432a	7735a	7738a	9707a
$25_{compost}$ + $75_{effluent}$	4437b	6621b	7847a	9074b
$50_{compost}$ + $50_{effluent}$	4167b	5218c	7137b	7924c
$75_{compost}$ + $25_{effluent}$	3641c	4594d	6655c	7440d
$100_{compost}$ + $0_{effluent}$	3592c	3500e	6208d	6381e

In the lysimeter experiment, irrespective of the soil types, total DM yield (mean of the two soil types) was significantly higher for the treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$). In this experiment, total DM yield for the treatment with effluent-N alone (($0_{compost}$+$100_{effluent}$)) was significantly lower than for treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) despite the former supplying more readily available N through STSE. As reported in **Chapter 5**, total N supplied through STSE for the treatment, ($0_{compost}$+$100_{effluent}$) was less than 150 kg N ha^{-1} like the other combinations of compost and sewage STSE. However, analysis of cumulative NO_3^--N loss through leaching **(Section 6.5.1)** showed that the loss of NO_3^--N from the treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) was significantly higher ($p < 0.05$).

In relation to N application rate, the study has found that ryegrass DM yield significantly increased with increasing N application rate from 75 to 150 kg N ha^{-1}. Grass crops have been reported to respond linearly to N application rates within the range of 0 to 300 kg N ha^{-1} (Antille, 2011; Morrison et al., 1980). At all N application rates, the combinations of compost and STSE were linearly related to each other and to the DM yield. At N application rate of 75 kg N ha^{-1}, the rate of decline for every addition of compost in a combination of compost and STSE was *c*.550 kg DM ha^{-1} as compared to *c*.323 kg DM ha^{-1} for the second year. Similarly at 150 kg N ha^{-1}, the rate of decline of DM yield was higher in 2011/12 (*c*.1166 kg DM ha^{-1}) as compared to the first year (713 kg DM ha^{-1}).

6.4.2 Nitrogen uptake and N in plant material

Nitrogen uptake (N_{uptake}) for 2010/11 and 2011/12 for the pot experiment showed that it was significantly influenced by soil type, N application rates and the combinations of compost and STSE. In both experiments, estimated N_{uptake} was influenced by the soil types. Actually, despite non-significant difference among the combinations of compost and STSE in the lysimeter experiment; in the pot experiment the combinations of compost and STSE influenced N_{uptake} in both soil types ($p < 0.05$). The environmental difference between the glasshouse and lysimeter (field) influenced the STSE irrigation regime and also the uptake of nitrogen. Mean N_{uptake} was higher in treatments without any contribution of compost-N ($0_{compost}$+$100_{effluent}$). With each addition of compost contribution, N_{uptake} declined. This research has shown that with time, N_{uptake} in the treatment with least contribution of compost (($25_{compost}$+$75_{effluent}$)) can perform just like the treatment with STSE alone (($0_{compost}$+$100_{effluent}$)).

TN_{plant} increased with N application rate which was in conformity with a study by Aavola and Kärner (2008). Reduced fertilisation has a negative effect on sward quality through reduced herbage digestability, compromised growth rate, reduced tiller density and biomass production (Aavola and Karner, 2008; Delagarde et al., 1997). Overall from the pot experiment, TN_{plant} decreased in the order ($0_{compost}$+$100_{effluent}$) < ($25_{compost}$+$75_{effluent}$) < ($50_{compost}$+$50_{effluent}$) < ($75_{compost}$+$25_{effluent}$) < ($100_{compost}$+$0_{effluent}$). But despite the higher N_{uptake} obseved in the pot experiment, TN_{plant} did not significantly differ between the various combinations of compost and STSE. Aavola and Kärner (2008) reported that frequent defoliation (cutting) of ryegrass enhances N content of ryegrass. However, the concentration of nitrogen in ryegrass herbage for the combinations of compost and STSE was above the minimum requirement for N in herbage for productive grazing animals of 20 g N kg^{-1} DM (Wilkins et al., 2000). But most combinations of compost and STSE fell short of the requirement for higher producing dairy cows for N herbage of between 2.2 – 2.7% (Aavola and Karner, 2008), apart from the treatments ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$).

6.4.3 Nitrogen use efficiency

NUE using PFP_e was significantly influenced by the soil type, N application rates and the combinations of compost and STSE. During the two year pot experiment, PFP_e was significantly higher in the clay loam soil (75 kg DM kg^{-1} applied N) as compared to the sandy loam (47 kg DM kg^{-1} applied N). NUE declined significantly with increasing contribution of compost. The highest PFP_e (73 kg DM kg^{-1} applied N) was recorded in the treatment ($0_{compost}$+$100_{effluent}$) while the treatment ($100_{compost}$+$0_{effluent}$) had lowest PFP_e of 49 kg DM kg^{-1} applied N. Similarly in the lysimeter experiment, PFP_e was higher in the treatment ($0_{compost}$+$100_{effluent}$).

It is possible to increase PFP_e by increasing the amount, uptake and utilisation of indigenous N resources and by increasing the efficiency with which applied N is taken up by the crop and utilised to produce yield (Cassman et al., 1998). As reported in **Chapter 4,** PFP_e was significantly higher at N application rate of 75 kg N ha^{-1} as compared to 150 kg N ha^{-1}. Similarly Hussain et al., (1996) observed that NUE declined with increasing N application. Actually they found higher NUE using PFP_e in control treatment with no application of nitrogen. Aavola (2005) concluded that at every increase in N application rates, NUE decreased. A higher PFP_e can sometimes result in unacceptably low DM yield production (Zhu et al., 2011). Nutrient use efficiency is higher at a low yield level, because any small amount of nutrient applied could give a large yield response (Dibb, 2000; Roberts, 2008). Despite higher NUE at N application rate of 75 kg N ha^{-1}, DM yield was unacceptably low such that it could not reach 90% of maximum ryegrass yields. The shortfall of PFP_e as an estimate of NUE is that since it is an aggregate efficiency index, it includes contribution to DM yield derived from uptake of indigenous soil nitrogen.

Matching N mineralisation to the pattern of growth of ryegrass can also help to improve NUE. The rate at which ryegrass regrowth occurs after cutting is influenced by the maturity of ryegrass at harvest such that there is a period of slow growth after defoliation (Fischer and Jewkes, 2009). As such after each ryegrass cut, N deficiencies are likely to occur as N loss is higher due to plant uptake and the demand for N is higher for regeneration (Beard, 1972).

Considering the low N mineralisation rates associated with compost, matching N requirements to plant growth cycle can be achieved in the presence of STSE. About 82% of dissolved total N in the STSE used was in mineral form (NH_4^+-N and NO_4^--N), readily available for plant uptake. If N application rate is low e.g. 75 kg N ha^{-1} or the contribution of STSE is low e.g. ($75_{compost}$+$25_{effluent}$), the period to irrigate with STSE is short (to meet the required effluent-N supply). For example, STSE irrigation for the treatment ($75_{compost}$+$25_{effluent}$) in the lysimeter experiment was from May to July 2011. The second to fourth ryegrass cuts for the ($75_{compost}$+$25_{effluent}$) treatment were made after cessation of effluent irrigation. As discussed above, ryegrass N requirement is higher after cutting. And in the absence of effluent-N, regrowth of ryegrass tillers was likely affected as effluent provided readily available N.

6.5 Environmental impact

6.5.1 Nitrogen

Cumulative loss of NO_3^--N in the clay loam through leaching was higher in treatments ($0_{compost}$+$100_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$). The treatment with greenwaste compost alone, ($100_{compost}$+$0_{effluent}$) had the lowest loss of NO_3^--N from the soil. The cumulative loss of NO_3^--N for the treatments ($25_{compost}$+$75_{effluent}$) and ($100_{compost}$+$0_{effluent}$) was not significantly different. Nitrate leaching from soils amended with organic products depends on the nature of material, the applied dose, the time of application, the quantity of water applied, the type of soil and in the case of cultivated soils, can also depend on N uptake by the crop (Burgos et al., 2006).

The low nitrogen leaching loss from the treatment ($100_{compost}$+$0_{effluent}$) was probably a reflection of the amounts of NO_3^--N available when compost alone is applied in soil. In the sandy loam, only 0.4% of the total applied N in was lost through leachate. This was in agreement with observation made in the incubation experiment (**Chapter 3**). While the lower cumulative NO_3^--N loss for the treatment ($25_{compost}$+$75_{effluent}$) in the clay loam was as a result of the higher mean DM yield for this treatment. NUE in both experiments was higher for the treatment ($0_{compost}$+$100_{effluent}$) followed by the treatment

($25_{compost}+75_{effluent}$). Morrison et al., (1980) reported that leaching losses of nitrogen from grass are unlikely until the quantity of applied nitrogen is excessive in terms of yield response. However, ryegrass is shallow rooted, with up to 80% of its roots in the top 15 cm of soil and has limited ability to intercept nitrate before it is leached (Dunbabin et al., 2003; Haynes and Williams, 1993; Moir et al., 2012).

Loss of N to the environment occurs in late season when grass growth is declining (Fischer and Jewkes, 2009). Fischers and Jewkes (2009) recommended that in the UK, no N application is done after mid-August and application in early August should be kept at a maximum of 50 kg N ha^{-1}. In the research study, for treatments with higher contribution of STSE e.g. ($0_{compost}+100_{effluent}$) and ($25_{compost}+75_{effluent}$), irrigation with STSE continued as long as estimated evapotranspiration losses were above rainfall amount to satisfy N supply from STSE; thereby increasing the susceptibility of N to leaching losses as grass growth is minimal during this period. This presents a challenge to the management of combined application of compost and STSE.

In the sandy loam soil, mean cumulative NO_3^--N leaching was lower (0.49 kg NO_3^--N ha^{-1}) as compared to 15.5 kg NO_3^--N ha^{-1} in the clay loam. Potential N mineralisation in the sandy loam from the incubation experiment was -0.17 kg inorganic N kg^{-1}. As mentioned earlier on, N immobilisation or low N mineralisation in the sandy loam likely influenced N availability. The end result was that leaching losses of NO_3^--N in the sandy loam for the combinations of compost and STSE were not significantly different ($p > 0.05$) to each other in the lysimeter experiment.

Using the approach of Honneycutt et al., (1991) and Smith et al., (1998) of thermal units (d°C; base temperature = 0°C), a possible link between the results of laboratory incubation and cumulative NO_3^--N leaching (lysimeter experiment) can be established. The approach normalises the effect of temperature and time (days) by considering them as a combined factor. The highest concentration of NO_3^--N for the treatment with effluent alone (in the clay loam) in the incubation experiment ($0_{compost}+37.5_{effluent}$), was at an accumulated thermal time of between 1550 to 2250 d°C. Using the mean monthly temperature reported in **Chapter 5**, similar accumulated thermal time was observed between July and August 2011 in the lysimeter experiment. In July 2011, cumulative loss of NO_3^--N for the treatment with effluent alone, ($0_{compost}+100_{effluent}$) was *c*.18 kg

NO_3^--N ha^{-1}. At the same time, cumulative NO_3^--N ha^{-1} leaching was 10 kg NO_3^--N ha^{-1} for the treatments, ($50_{compost}+50_{effluent}$) and ($75_{compost}+25_{effluent}$). NO_3^--N leaching from soils amended with organic products depends on the nature of material, the applied dose, the time of application, the quantity of water applied, type of soil and in the case of cultivated soils, also can depend on N uptake by the crop (Cabrera et al., 2005).

The drinking water standard (10 mg NO_3^--N l^{-1}) is likely to be exceeded in combinations of compost and STSE in the clay loam soil. Excess NO_3^--N in potable water can lead to infant mortality resulting from a reduction of NO_3^- to NO_2^- by microorganisms in children stomachs and in the rumen of animals (Fonseca et al., 2007b). The mean concentration of NO_3^--N for the combinations of compost and STSE was significantly higher in the clay loam (11.48 mg l^{-1}) as compared to the sandy loam soil (1.78 mg l^{-1}). In the sandy loam, peak NO_3^--N concentration was observed for the treatment ($25_{compost}+75_{effluent}$) and ($50_{compost}+50_{effluent}$). In the sandy loam, the threat to the health of human beings from these treatments can be considered mild as the peaks above the drinking water standard were limited as compared to the number of leachate samples collected during the duration of the experiment. The drinking water standard limit was exceeded three times of the 16 times leachate was sampled. In the clay loam soil peaks of higher NO_3^--N concentration were recorded largely during the first four months of the experiment after which the concentration of NO_3^--N in leachate was below the drinking water standard. The first four months of the lysimeter corresponded with the period in which larger quantities of STSE were irrigation to the ryegrass.

6.5.2 Phosphorous

The combinations of compost and STSE did not influence the loss of PO_4^{3-}-P through leaching. Similarly the loss of PO_4^{3-}-P in leachate was not different between the two soils types. Phosphorous combines with iron and aluminium which according to Troeh and Thompson (2005) results in compounds that are mostly nearly insoluble. This characteristic, not only does it seriously limit availability of P in the soil, it also helps to keep leaching losses of P very small.

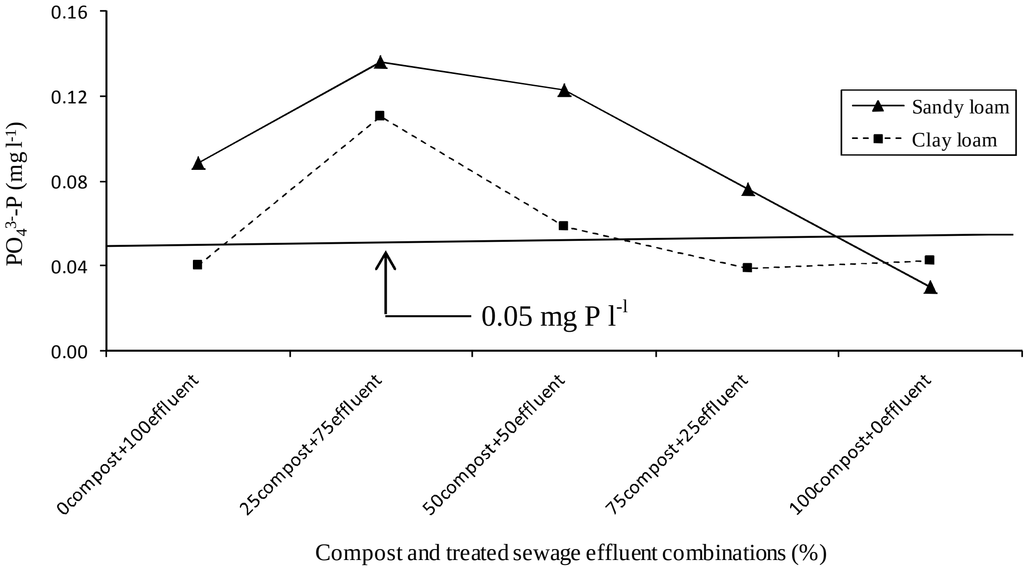


Figure 6-3 Mean concentration of PO_4^{3-}-P in leachate for the duration of the lysimeter experiment (p = 0.69).

The after effects of PO_4^{3-}-P leaching are detrimental to the environment especially in water bodies. Additional loading of P in any of the various forms; orthophosphate, pyrophosphate, metaphosphate, mono and di-hydrogen phosphate, etc., results into an undesirably extensive growth of algae and/or other aquatic plants like water hyacinth (Khan and Ansari, 2005). When conditions are favourable, concentrations of P exceeding 0.05 mg l^{-1} may stimulate growth of algae and other aquatic plants in water bodies (Hinesly and Jones, 1990). The mean concentration of PO_4^{3-}-P in leachate has been presented in **Figure 6-3** for the combinations of compost and STSE in the sandy loam and the clay loam soils. Despite not showing any significant difference in terms of the interaction of soil type and combinations of compost and combinations of compost and STSE, most treatment combinations were above the limit of 0.05 mg l^{-1} for P concentration. The exceptions in the clay loam soil were ($0_{compost}$+$100_{effluent}$), ($75_{compost}$+$100_{effluent}$) and ($100_{compost}$+$0_{effluent}$) treatments, while PO_4^{3-}-P concentration was 0.11 and 0.06 for treatments ($25_{compost}$+$75_{effluent}$) and ($50_{compost}$+$50_{effluent}$) respectively. In the sandy loam, apart from the treatment with compost alone ($100_{compost}$+$0_{effluent}$), the mean concentration of PO_4^{3-}-P for all the other combinations of compost and STSE was above the 0.05 mg l^{-1} limit.

The risk of water eutrophication from combined application of compost and STSE present a challenge to the integration of compost and STSE. As reported in **Chapter 4 and 5**, mean total P and PO_4^{3-}-P concentration in the STSE used for the research study was 6.2 and 5.9 mg l^{-1} respectively while the greenwaste compost had 2.1 and 2.8 g kg^{-1} for the lysimeter and pot experiments respectively. Eghball and Power (1999) in a 4-year field experiment concluded that nitrogen-based compost application results in soil phosphorous levels significantly higher than those for P-based fertiliser application. Compost has been reported to effectively supply P to soil with soil P concentration increasing with increasing application rates (Iglesias-Jimenez and Alvarez, 1993). The amendments in the experiments (pots and lysimeter) were made on soils with higher P concentration of 0.51 and 0.47 g kg^{-1} for the sandy loam and the clay loam soil respectively in the lysimeter experiment and 0.84 and 0.64 g kg^{-1} for the sandy loam and the clay loam soil respectively in the pot experiment.

In other words, even before STSE irrigation, the soils had enough reserves of P to meet plant P requirements. STSE irrigation increased the solubility of P in the soil and its susceptibility to leach. This explains why in most treatments with combination of compost and STSE, the risk to eutrophication can be higher as the concentration of PO_4^{3-}-P in leachate was above the limit of 0.05 mg l^{-1}. Clearly, integration of compost and STSE on soils rich with P can create severe threat of water eutrophication.

6.6 Optimum integration of STSE and compost nutrient

Following the results presented from **Chapter 3** to **5** and the Integrated Discussion, this section will bring together and summarise some of the key outcomes presented and discussed for determination of optimum combination of compost and STSE. A summary of the key outcomes has been outlined in a thermometer-styled chart in **Figure 6-4**.

Increasing the contribution of STSE while reducing compost quantity resulted in increased nitrogen use efficiency (NUE) and ryegrass dry matter yield (DM). The increase in DM yield and NUE was of the order ($0_{compost}$+$100_{effluent}$) > ($25_{compost}$+$75_{effluent}$) > ($50_{compost}$+$50_{effluent}$) > ($75_{compost}$+$25_{effluent}$) > ($100_{compost}$+$0_{effluent}$). At 75 kg N ha^{-1}, DM yield for the treatments ($0_{compost}$+$100_{effluent}$) and ($25_{compost}$+$75_{effluent}$) was not significantly different while at N application of 150 kg N ha^{-1}, DM yield was significantly higher for the treatment ($0_{compost}$+$100_{effluent}$) but by only 6.5% in relation to the treatment ($25_{compost}$+$75_{effluent}$).

NM_{net} decreased with increasing proportion of compost in the clay loam soil. Mean NM_{net} in the clay loam soil for the incubation experiment was 1.6, 0.87, 0.36, 0.41 and 0.26 kg inorganic N kg^{-1} applied N for the treatments ($0_{compost}$ + $37.5_{effluent}$), ($35.7_{compost}$ + $37.5_{effluent}$), ($112.5_{compost}$ + $37.5_{effluent}$), ($75_{compost}$ + $0_{effluent}$) and ($150_{compost}$ + $0_{effluent}$) respectively. Increasing the contribution of compost in combined application of compost and STSE, with time resulted in a slow increase of net N mineralisation in the sandy loam. Treatments with higher NM_{net} in the clay loam had higher DM yield, NUE and higher cumulative loss of NO_3^--N. In the sandy loam, low NM_{net} resulted in low DM yield, NUE and less cumulative loss of NO_3^--N. However the consequence of increased N mineralisation in treatments with effluent-N in the clay loam was the increased risk of N leaching.

As presented in **Figure 6-4**, the threat to eutrophication due to leaching of P was enhanced with increased STSE contribution. The effect was also related to soil textural characteristics. In the research study, the treatment with compost alone, ($100_{compost}$+$0_{effluent}$) leached less P. The concentration of P in leachate reduced with inclusion of compost in compost and STSE nutrient integration (($25_{compost}$+$75_{effluent}$) > ($50_{compost}$+$50_{effluent}$) > ($75_{compost}$+$25_{effluent}$)).

As was mentioned in **Section 6.2**, application of STSE alone e.g. ($0_{compost}$ + $100_{effluent}$) contributed dissolved organic carbon which was essential in the short term as a source of energy to microbes. Inclusion of compost guarantees continuous build-up of carbon stock in the soil in long term for improved soil and crop productivity. Considering the future health of the soil, addition of extra carbon in the treatments ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) could be essential for sustainable soil processes.

In terms of heavy metals accumulation in the soil, increasing or decreasing compost and/or STSE did not have any significant bearing on heavy metals in short term. However for any combination of compost and STSE, regular soil monitoring will be essential to prevent future build-up of heavy metals in the soil.

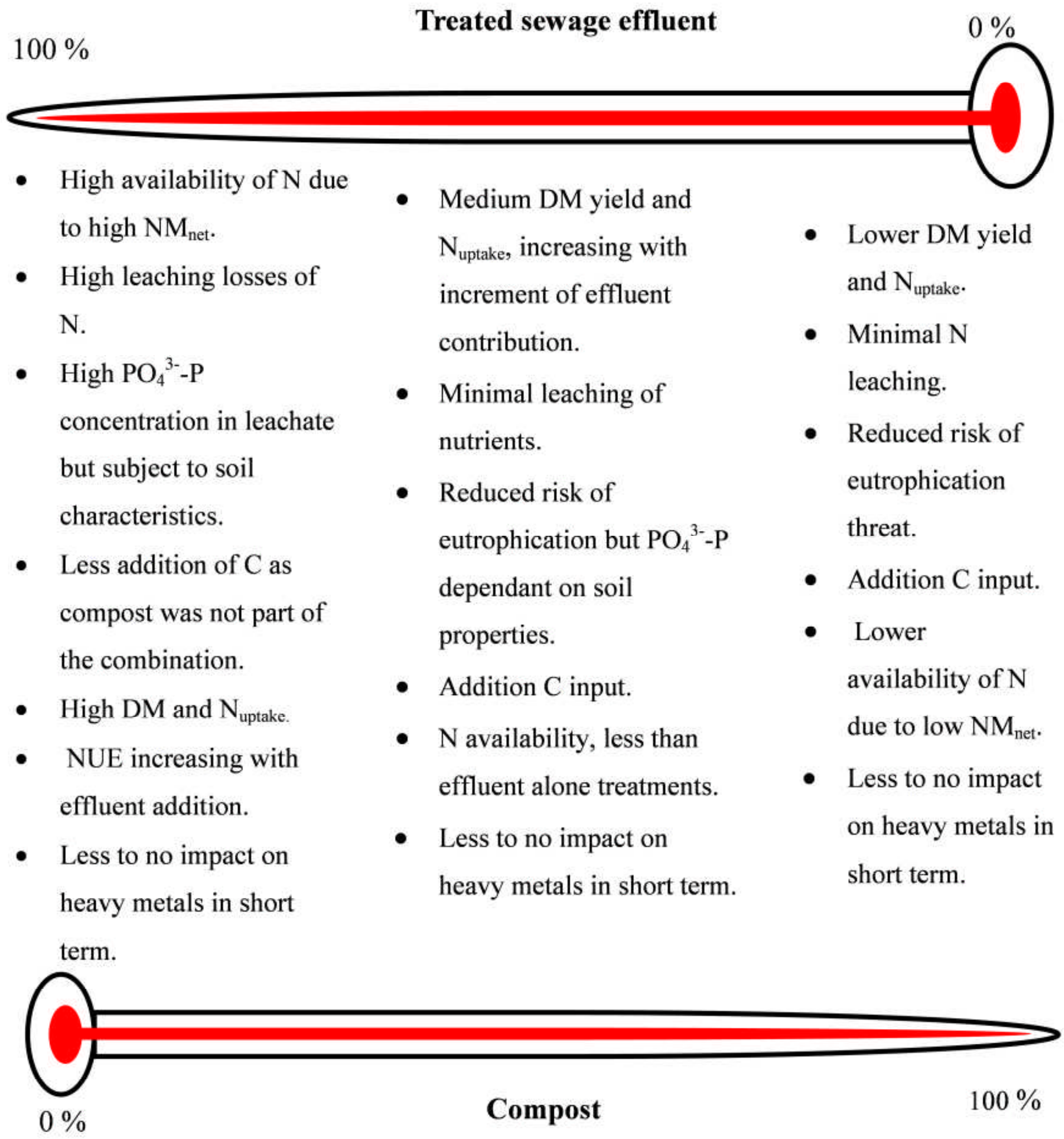


Figure 6-4 Summary of factors to consider when selecting optimum combinations of compost and STSE.

The results summarised in **Figure 6-4** and the accompanied discussion has shown that for optimal results and for future crop and soil productivity, the treatment ($25_{compost}$+$75_{effluent}$) is the ideal combination of compost and STSE. The treatment ($25_{compost}$+$75_{effluent}$) combines 25% compost with 75% STSE. The performance of this treatment in the pot and lysimeter experiments in both soil types has been the same or slightly less than the treatment with effluent alone ($0_{compost}$+$100_{effluent}$). Despite less DM yield for example, the build-up of carbon stock can be enhanced by the contribution of compost while the STSE can guarantee supply of readily available nutrients in the soil. This can ensure supply of nutrients in both short and long term. Actually, cumulative loss of N through leaching was lower for the ($25_{compost}+75_{effluent}$) as compared with the treatment with effluent alone.

6.7 Management and application of the research findings to developing countries

In this section a discussion will be made on practical issues that have to be considered for nutrient integration of compost and STSE and application of the study in developing countries. The considerations will include the characteristics of the STSE in relation to the health of the irrigators/farmers, choice of crops to grow and soil protection.

Application of the findings of this research study in developing countries is essential for improved food security and soil fertility. The traditional way to overcome nutrient depletion in arable soil is through the use of inorganic fertilisers. In most developing countries, widespread use of inorganic fertilisers by smallholder farmers is constrained by market price of fertilisers. In Malawi for example, with an average market price of £25 for a 50 kg bag of inorganic fertiliser, most resource poor smallholder farmers cannot afford to buy inorganic fertilisers. The UNDP (2011) estimates that 74% of the population in Malawi is below the income poverty line while 40% is in severe poverty. Fertiliser prices in Malawi are substantially higher than world prices and also higher than prices within the region (Malawi Government, 2006). Largely this is because Malawi is land locked which entails higher transportation costs, higher finance charges, limited competition and higher exchange rate risks (World Bank, 2004).

Since 2005/06 growing season, the Government of Malawi introduced the Farm Input Subsidy Program (FISP). Under FISP, inorganic fertiliser is sold to resource poor farmers, at a subsidised price of £1.27 for a 50 kg bag (Malawi Government, 2012). In the 2011/12 budget, about 6.7% of the total national budget was allocated to procure 140 000 metric tons of fertiliser which benefited 1.4 million farm families (Malawi Government, 2011). In the 2012/13 national financial budget, about 10% of the total national budget was allocated to procure 150 000 metric tons of fertiliser (Malawi Government, 2012). Although FISP has proved beneficial, it is not sustainable as it has continuously drained foreign exchange currency and diverted resources from others sectors.

The scenario presented above gives a gloomy picture about smallholder farmers in Malawi and the Sub Saharan Africa region as they are resource poor relying on agriculture as a source of livelihoods. Soil degradation caused by agricultural intensification and a general decline in soil fertility due to continuous cultivation of land remains the greatest threat to food supply to most countries in the Sub Saharan region. Organic amendments (animal manures, household composts, crop residues, leguminous cover crops, to leguminous and non- leguminous trees and shrubs) are often used as major nutrient sources to crops (Chivenge et al., 2011). However, low availability of materials has limited the use of some of these organic amendments. Most smallholder farmers commonly use crop residues as firewood or bedding to livestock. In some cases, farmers burn crop residues to reduce labour demands for land clearing. This has resulted in very high average annual depletion rates of 22 kg of nitrogen (N), 2.5 kg of phosphorous (P) and 15 kg of potassium (K) per hectare of cultivated land for the last 3 decades (Sanchez, 2002).

The focus and motivation for this research presented in **Chapter 1** was to offer a possible solution that can be applied to solve problems of soil fertility decline largely in developing countries. In most developing countries, smallholder farmers cannot afford to purchase inorganic fertilisers to replenish soil fertility and improve crop yield. Application of the findings of this research especially in developing countries will help in improving soil fertility. The resource poor peri-urban farmers will be able to cut costs by using compost and STSE. However, a number of considerations will have to be taken into account before adoption and management of the compost and STSE nutrient integration.

6.7.1 Suitability of STSE

Adoption of integrated compost and STSE nutrient application should focus not only on crop production but also on environmental protection. The main source of concern in integrated compost and STSE application is the STSE. This research study has shown increased concentration of phosphorous in leachate as a result of integrated compost and

STSE application. The source of STSE (municipal or industry) and the level of treatment can negatively impact on the soil in the long term.

The results presented and discussed in this research study showed advantages of combining STSE with compost in terms of nutrient supply and the lack of accumulation of heavy metals and nutrients in the short term if STSE of similar characteristics is used. From the analyses conducted on the STSE reported in **Chapter 4** and **5**, the electrical conductivity ranged from 764 to 862 µS cm^{-1}, NO_3^--N between 27 to 39 mg l^{-1} and pH was 6.7 to 7.0. Compared to the FAO classification (Ayers and Westcot, 1985) presented in Literature Review (**Chapter 2)**, the STSE was classified as having none to slight to moderate restriction for agricultural use. But, salinity build-up due to irrigation with poor quality recycled water is a constant threat especially in arid areas. Besides affecting crop yield and soil physical conditions, irrigation water quality affects fertility needs, irrigation system performance and longevity and how the water can be supplied (Ayers and Westcot, 1985; Elberling et al., 2003).

The guidelines presented in **Chapter 2** by Ayers and Westcot (1985) are generic hence a need to verify with Maas (1984) to assess suitability of the crop to be grown for the given effluent characteristics. Crop selection in relation to characteristics of STSE has been discussed in **Section 6.7.2**.

Due to higher costs of treatment processes and lack of effective environmental pollution control laws or law enforcement, there are very few wastewater treatment facilities in most developing countries (Kivaisi, 2001). This is why stabilisation ponds are widely used due to low installation and maintenance costs. Public agencies in many developing countries have limited ability to invest in or even maintain wastewater treatment plants and programs to optimise wastewater reuse. In most cases, the resultant is reduced efficiency of wastewater treatment (Qadir et al., 2010)

Reduced efficiency of waste water treatment plants affects suitability of effluent for agricultural production. Inefficiencies in waste water treatment in Malawi for example (Kauma Treatment Plant), have resulted in total N and total P in STSE above the WHO recommended limits (Mtethiwa et al., 2008). This is why Chiou (2008) concluded that total N content of reclaimed water is always too high for normal crop growth and recommended dilution to increase suitability of the reclaimed water for crop production.

Wastewater suitability for combined application with compost should consider the health risk associated with farmers who will be in contact with the STSE and the consumers. Risks associated with consumers have been discussed in **Section 6.7.2**. The inefficiencies mentioned above in waste water treatment in developing countries can result in STSE with health hazards and risks to irrigators. As mentioned in **Chapter 2**, STSE contains pathogenic microorganisms like bacteria, viruses, fungi, algal, etc., having the potential to cause diseases and create immense harm to public health. The water borne diseases associated with wastewater includes typhoid, paratyphoid fevers, dysentery and cholera, polio and infectious hepatitis.

6.7.2 Crop Selection

In the research study, perennial ryegrass (*Lolium Perenne*) was used as a test crop. Perennial ryegrass was described by Ayers and Westcot (1985) as moderately tolerant to salt levels. STSE used in combination with compost in both pot and lysimeter study was within the allowable margin of ryegrass in terms of electrical conductivity.

As reported in **Chapter 5**, for treatments supplying effluent-N alone, ($0_{compost}$+$100_{effluent}$) in the clay loam and the sandy loam soils and ($25_{compost}$+$75_{effluent}$) in the sandy loam soil, the projected quantities of effluent required were not met. Instead of supplying 455 mm for the ($0_{compost}$+$100_{effluent}$) treatment; 285 mm and 365 mm was supplied in the sandy loam and the clay loam soils respectively. As mentioned in **Chapter 5**, this was due to excessive rainfall during the experimental period. Similarly for the treatment ($25_{compost}$+$75_{effluent}$) in the sandy loam, instead of 341 mm, 297 mm was supplied. This actually meant that less total N was supplied for these treatments.

In the Sub-Saharan Africa region, maize (*Zea mays*) is the commonly grown cereal for commercial and consumption. It is the major staple crop occupying about 80% of the land area under cultivation (Ito et al., 2007) and accounting for more than 80% of the population's caloric intake (Denning et al., 2009). The growth period for early maize grain varieties is 80 to 110 days and medium varieties, 110 to 140 days to mature while N requirement is up to about 200 kg N ha^{-1} N for high-producing varieties (FAO, 2012).

Table 6-4 Estimated quantity of STSE and compost for a crop with short growing season (maize) to 200 kg N ha^{-1}.

Combination of compost and STSE (%)	Estimated effluent irrigation depth (mm)	Estimated compost requirement (t ha^{-1})
$100_{compost} + 0_{effluent}$	0	12.1
$75_{compost} + 25_{effluent}$	152	9.1
$50_{compost} + 50_{effluent}$	303	6.1
$25_{compost} + 75_{effluent}$	455	3
$0_{compost} + 100_{effluent}$	606	0

Table 6-4 shows estimated quantities of compost and STSE assuming the test crop was maize (*Zea mays*) instead of the perennial ryegrass. Considering the growing season of the crop (maize), for the treatments ($0_{compost}$+$100_{effluent}$), farmers will likely fail to supply the required quantity of 606 and 455 mm respectively through STSE in 110 to 140 days. Length of the growing season is a factor to consider when integrating compost and STSE. In the glasshouse, because of the controlled environment that guaranteed no effective rainfall, it was possible to supply the required amounts of STSE. It was not the case with the lysimeter experiment where irrigation depth was defined by the difference between estimates of evapotranspiration and rainfall amount. Clearly, success of the compost-effluent nutrient integration will also depend on crop selection.

Emongor and Ramolemana (2004) recommended growing forage crops under STSE irrigation because of their longer growing season, higher evapotranspiration demand and removal of large quantities of nutrients from the system. The choice of forage crops takes into consideration the health of consumers as forages are not directly consumed by humans. Successful implementation of compost-effluent nutrient integration can therefore be affected by acceptance of crops grown under STSE. There will always be some doubt about the safety of water reuse for agriculture, so rational criteria for reclaimed water on the basis of a reliable health risk assessment has to be considered (Chiou, 2008). Chemical risks of using reclaimed water for agricultural irrigation might occur by eating polluted crops and livestock, or by drinking and being in contact with reclaimed water (Chiou, 2008; Shuval et al., 1997; Stewart, 2006).

Policy establishment to moderate and regulate usage of treated effluent either alone or in combination with other nutrient sources is essential to safeguarding the health of irrigators and consumers. There is a need to better integrate water reuse into core water governance frameworks in order to effectively address the challenges and harness the potential of this vital resource for environmental health protection (Hamdi et al., 1994). Through policies, controls can be set on what crops to be grown whenever STSE is involved. For example, in some South American countries, treated effluent either alone or in combination with other nutrient sources cannot be applied to lettuce, cabbage, beets, coriander, radishes, carrots spinach and parsley (Emongor and Ramolemana, 2004). Policies to reduce the negative impacts of wastewater usage while supporting its benefits can target the situations before the wastewater is generated, while it is being used and after crops have been irrigated and products are prepared for sale and consumption (Qadir et al., 2010). Unfortunately most developing countries (especially in Sub Saharan Africa) have no policies to guide on agricultural utilisation of wastewater which is why in Accra (Ghana) for example, about 1000 farmers supply the urban street food sector with lettuce nearly all of which is contaminated (Qadir et al., 2010).

6.7.3 Irrigation system/methods

The health risks associated with usage of STSE either in combination with compost or alone, increases as the irrigators/farmers get in contact with STSE. Selection of irrigation method is significant to reduce human contact with effluent, without affecting supply of water and nutrients to plants. The decision by farmers on which irrigation method to adopt does not only depend on water quality but also on affordability, tenure security, labour availability and other production factors (Qadir et al., 2010).

In both experiments (pots/glasshouse and lysimeter), STSE was irrigated manually using a watering can. The possibility of using drip irrigation system was set aside in the study. The narrow passages in the drippers (drip irrigation system) make them vulnerable to blocking by suspended particles and algae (Myers et al., 1999; Minhas and Samra, 2004). Blocking of drippers can reduce the flow rate of STSE and lead to under-irrigation of STSE.

Wang (1997) used a two-dimensional solute transport model to investigate the comparative effects of sprinkler, drip and furrow irrigation. He concluded that sprinkler irrigation was the least likely to cause ground water contamination. Sprinkler irrigation systems can produce a regular and unimodal wetting front (Livesley et al., 2007; Hamdi et al., 1994). But the nature of sprinkler and micro-sprinkler irrigation makes these methods less appropriate to control health and contamination hazard as well as toxicity hazards (Pereira et al., 2002).

As discussed in **Chapter 5**, preferential flow was a greater concern in the lysimeter experiment especially because of using disturbed/repacked soil. Preferential flow can result in over-estimation of leachate quantities and under estimation of nutrient leaching as the leachate does not get in contact with soil. Preferential flow has been linked to irrigation methods. In preferential flow studies comparing ponding and non-ponding irrigation systems (e.g., flood irrigation and sprinkler irrigation), it has been reported that ponding irrigation systems promote preferential flow (Livesley et al., 2007; Chen et al., 2002). However, in most developing countries due to capital costs associated with installation of sprinkler and drip irrigation system (pressurised irrigation systems); ponding irrigation systems (furrow and basin irrigation systems) are popular amongst smallholder farmers (**Chapter 2**) in developing countries.

This means that for compost and STSE nutrient integration, smallholder farmers will likely use ponding irrigation methods with a variety of water lifting appliances. This will increase contact with STSE. It is therefore paramount that the STSE should meet standards set to guarantee healthy safety of the smallholder farmers and also protect the environment.

7 CONCLUSIONS AND RECOMMENDATIONS

This chapter summarises the overall conclusions coming from the research study. The aim and objectives of the research were already presented in **Chapter 1.** The detailed main conclusions from each of the experiments have been presented from **Chapter 3 to 5** and the integrated discussion in **Chapter 6**. The conclusions and the areas for further research drawn from the experimental results and analysis are presented below.

7.1 Overall conclusions

The main conclusions which can be drawn from the research study are as follows;

1. The interaction of nutrients in combined application of compost and STSE is controlled by different mechanisms that depend of the physical and chemical characteristics of the soil and STSE. Multiple mechanisms influenced the dynamics of nitrogen in the soil as a result of compost and STSE integration. Cation exchange capacity, quality of available carbon, soil microbial activity and drying and rewetting cycles of soil are the mechanisms involved in nutrient interaction of compost and STSE nutrients. The mechanisms influenced release and availability of nitrogen and impact of the combinations of compost and STSE on crop production and leaching of nutrients.

   The influence of the above mechanisms of nutrient interaction on the availability of nitrogen in the soil was that;

   - Release of NO_3^--N was higher in integrated compost and STSE nutrient combinations in the clay loam (0.71 kg inorganic N kg^{-1} applied) as compared to the sandy loam soils (-0.17 kg inorganic N kg^{-1} applied). Immobilisation of N in the sandy loam mainly in treatments with STSE alone and combinations of compost and STSE affected NO_3^--N dynamics.
   - Overall net N mineralisation was significantly higher (1.6 kg inorganic N kg^{-1} applied N) in treatments with effluent alone (($0_{compost}$ + $37.5_{effluent}$)) in the clay loam soil. Increasing the quantity of compost in combinations of compost and STSE resulted in reduced net N mineralisation. In the clay loam, net N mineralisation in treatments with STSE alone and

combination of compost and STSE was higher than the applied N, suggesting a possibility of rapid microbial growth and higher microbial activity in clay loam soil.

2. The environmental threat to ground and surface water pollution through NO_3^--N leaching may be enhanced by the inclusion of STSE in integrated compost and STSE nutrient supply to plants. Overall, the threat from NO_3^--N leaching to the environment was greater in the clay loam soil as compared to the sandy loam soil. Mean NO_3^--N concentration was 11.5 and 1.8 mg l^{-1} for the clay loam and the sandy loam soils respectively. Peak concentrations of NO_3^--N of above the drinking water quality standard (10 mg l^{-1}) were observed mostly from treatments with effluent N alone, ($0_{compost}+100_{effluent}$) and combined application of compost and STSE, ($50_{compost}+50_{effluent}$) in the clay loam. In the sandy loam, susceptibility of NO_3^--N to leach was minimal as witnessed by the low concentration of NO_3^--N in leachate. Fewer peaks of NO_3^--N concentration in leachate were observed for the treatments, ($25_{compost}+75_{effluent}$) and ($50_{compost}+25_{effluent}$) in the sandy loam.

   Phosphate concentration in leachate was influenced by the combinations compost and STSE. The highest mean concentration of PO_4^{3-}-P in leachate was from the treatment ($25_{compost}+75_{effluent}$) of 0.12 mg l^{-1}. The difference of PO_4^{3-}-P concentration in leachate between the various combinations of compost and STSE was small. However, the threat to eutrophication is likely to be higher when combinations of compost and STSE are made on the sandy loam soil.

   - Mean concentration of phosphate for treatments, ($100_{compost}+0_{effluent}$), ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$) and ($75_{compost}+25_{effluent}$) in the sandy loam was above the limit that can result in eutrophication of 0.05 mg l^{-1}.
   - The treatment with compost-N alone, ($100_{compost}+0_{effluent}$) registered the lowest concentration of phosphate in leachate. The mean concentration of phosphate was 0.03 and 0.04 mg P l^{-1} for the ($100_{compost}+0_{effluent}$) treatment in the sandy loam and the clay loam soils respectively.

3. Ryegrass DM response to combined application of compost and STSE depends on the quantity of compost or STSE in a combination. Ryegrass DM yield

reduced with increasing contribution of compost (whilst reducing the amount of STSE irrigated). Overall, DM yield declined by 9, 26, 37 and 56% in the pot experiment due to addition of compost from the treatment ($0_{compost}+100_{effluent}$) to ($25_{compost}+75_{effluent}$), ($50_{compost}+50_{effluent}$), ($75_{compost}+25_{effluent}$) and ($100_{compost}+0_{effluent}$) respectively.

- The relationship between the combinations of compost and STSE and DM yield was better explained using linear equations at the two N application rates. At N application rate of 75 kg N ha^{-1}, the rate of decline for every unit addition of compost in a combination of compost and STSE was *c.*550 kg DM ha^{-1} as compared to *c.*323 kg DM ha^{-1} for the second year. Similarly at 150 kg N ha^{-1}, the rate of decline of DM yield was higher in 2011/12 (*c.*1166 kg DM ha^{-1}) as compared to 2010/11 (713 kg DM ha^{-1}).

- The concentration of N in ryegrass herbage for the combinations of compost and STSE was above the minimum requirement for N in herbage for productive grazing animals of 20 g N kg^{-1} DM. But most combinations of compost and STSE fell short of the requirement for higher producing dairy cows for N herbage of between 2.2 – 2.7%, apart from the treatments ($0_{compost}+100_{effluent}$) and ($25_{compost}+75_{effluent}$).

4. Integrated application of compost and STSE in short term will not result in significant changes in the levels of Cr, Cu, Ni, Pb and Zn in the soil if effluent of similar characteristics is used in combination with compost. The concentration of all the heavy metals was below the maximum permissible concentration of potential toxic elements. In long-term, it is essential to closely monitor the soil to ascertain that the build-up of metals does not go above the maximum permissible heavy metal limits.

    - In the short term, combinations of compost and STSE will not influence soil physical and chemical properties. The combinations of compost and STSE did not induce any significant change of total N (TN_{soil}) in the soil. Instead, TN_{soil} was affected by soil types in both experiments. Similarly soil extractable P was not influenced by integrated application of compost and STSE instead; it was significantly affected by N application rates and soil types.

- The analyses of SOM in the lysimeter experiment showed that there was an overall increase as compared with the initial levels. Compared to background values of SOM in the clay loam soil (5.7%) in the lysimeter experiment, mean SOM increased to 6.4, 6.2, 6.0, 6.1 and 6.2% for the treatment ($0_{compost}$+$100_{effluent}$), ($25_{compost}$+$75_{effluent}$), ($50_{compost}$+$50_{effluent}$) and ($75_{compost}$+$25_{effluent}$) and ($100_{compost}$+$0_{effluent}$) respectively.

In summary, this research has contributed knowledge by addressing an existing problem of declining soil fertility in a novel way through integration of compost and STSE. It has also determined the associated implications of integrated application of compost and STSE on nutrient dynamics, crop production, soil properties and leaching of nutrients.

- The research has shown that integrated compost and STSE has potential to provide plant nutrients and produce higher dry matter yield than when applying compost alone. In general, the impact and influence of integrated compost and STSE nutrient integration on crop production reduced with increasing compost quantity in combinations of compost and STSE.
- The under-lying potential mechanisms behind the release of N from treatments with compost and STSE nutrient integration has been cation exchange capacity, availability of carbon, soil microbial activity and rewetting and drying cycles of soil. These mechanisms have influenced the availability of nutrients as evidenced by differences in responses to dry matter production, nutrient use efficiency and leaching of nutrients due to the combinations of compost and STSE.
- One notable observation made from the study has been the threat of phosphorous leaching. Mean phosphorous concentration in leachate was above the limit of 0.05 mg l^{-1} for most combinations of compost and STSE in both soils. Closer observations are required for sustainable protection of the environment especially water bodies. In terms of NO_3^--N leaching, peaks above the drinking water standard of 10 mg l^{-1} were observed in the clay loam largely

for treatments with higher nutrient contribution from STSE. In the sandy loam, the low net N mineralisation reported in the incubation experiment meant that less NO_3^--N was available not only to the growing plants but also for potential leaching.

- The ideal approach to maximising nutrient potential from compost through irrigation with STSE is when 25% compost is combined with 75% STSE with respect to nitrogen supply. The performance of the treatment ($25_{compost}$+$75_{effluent}$) in the research study was either the same or slightly less than the treatment with effluent alone ($0_{compost}$+$100_{effluent}$) in most of the parameters tested in the study. The treatment ($25_{compost}$+$75_{effluent}$) can balance readily available nutrients in STSE to build-up of soil organic matter and carbon stock. Maintaining or enhancing carbon storage requires consistent input of carbon. The build-up of carbon stock can be enhanced by the contribution of compost while the STSE ensures supply of readily available nutrients to plants in the soil. This can ensure supply of nutrients in both short and long term. Total nitrogen in plant material for this treatment has satisfied the requirement for both grazing and dairy animals in terms of nitrogen in plant materials.

Adoption of the integrated compost and STSE nutrient application in other places e.g. Malawi, will be limited by the quality of the STSE. The quality of STSE is likely to be different amongst countries and between regions thereby affecting the usage of STSE for integrated nutrient application. As mentioned in **Chapter 6**, treatment inefficiencies of most sewage treatment plants in developing countries affect the quality of the STSE. In the case of low quality STSE, the number of crops potentially grown under the integrated compost and STSE will be less and the focus will likely shift to fodder, bioenergy and other commercial crops e.g. cotton.

7.2 Recommendations for further research

This research study has pioneered research in integrated soil fertility management through irrigation of STSE on soils amended with greenwaste compost. This research has highlighted areas which would require scientific research to further knowledge. The research focussed on short term impacts of integrated compost and STSE nutrient application on soil properties, crop production and leaching of plant nutrient. A longer term study on the effects of the integration is essential for better understanding of the future consequences of the compost-effluent nutrient integration especially on heavy metals, leaching and accumulation of plant nutrients in the soil. This will help to ensure environmental protection and improved nutrient availability to plants.

Determination of net N mineralisation showed potential mineralisation of native soil organic matter as evidenced by the higher N mineralisation in treatments with STSE alone, ($0_{compost}$+$37.5_{effluent}$) and combined compost and treated sewage application, ($37.5_{compost}$+$37.5_{effluent}$) in the clay loam soil. A detailed study is required to ascertain this potential phenomenon using isotopic labelling of nitrogen and carbon. Labelling different pools with ^{14}C and ^{15}N gives the possibility to state clearly the source of released C and N.

It is imperative to study and understand the microbial role in decomposition of organic matter due to the combined application of compost and STSE. As alluded to in this research and considering the shortfalls of fumigation-extraction methodology, other methods e.g. substrate induced respiration can be used provide an insight to the role, type (microbial diversity) and behaviour of microbes when compost is integrated with STSE.

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APPENDICES

Appendix A Chapter 3

A.1 Statistical analyses

Table A.1-1 Analysis of variance for net nitrogen mineralisation (NM_{net})

Source of variation	SS	D.o.F	MS	F	p
1) Soil type	28.7	1	28.7	7685	***0.00***
2) Mix + rate	6.0	4	1.5	398	***0.00***
Soil type*Mix + rate	14.3	4	3.6	959	***0.00***
Error	0.1	20	0.0		
3) TIME	2.1	4	0.5	2586	***0.00***
TIME*Soil type	8.5	4	2.1	10611	***0.00***
TIME*Mix + rate	2.0	16	0.1	625	***0.00***
TIME*Soil type*Mix + rate	4.8	16	0.3	1499	***0.00***
Error	0.0	80	0.0		

Table A.1-2 Analysis of variance for soil pH for soil samples in the incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	0.1	5	0.021	5	***0.00***
2) Soil type	3.1	1	3.092	717	***0.00***
Mix + rate*Soil type	0.0	5	0.007	2	0.18
Error	0.1	22	0.004		
3) TIME	0.9	3	0.295	52	***0.00***
TIME*Mix + rate	0.1	15	0.009	2	0.11
TIME*Soil type	0.4	3	0.137	24	***0.00***
TIME*Mix + rate*Soil type	0.1	15	0.008	1	0.18
Error	0.4	66	0.006		

Table A.1-3 Analysis of variance for total carbon for soil samples at the start and end of incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	0.03	5	0.006	1.24	0.32
2) Soil type	7.44	1	7.441	1534.34	***0.00***
Mix + rate*Soil type	0.01	5	0.002	0.50	0.77
Error	0.12	24	0.005		
3) TIME	0.02	1	0.025	7.89	***0.01***
TIME*Mix + rate	0.01	5	0.003	0.89	0.51
TIME*Soil type	0.00	1	0.002	0.68	0.42
TIME*Mix + rate*Soil type	0.02	5	0.003	1.08	0.40
Error	0.08	24	0.003		

Table A.1-4 Analysis of variance for total nitrogen for soil samples at the start and end of incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	0.00	5	0.00	1.37	0.27
2) Soil type	0.06	1	0.06	1147.83	***0.00***
Mix + rate * Soil type	0.00	5	0.00	1.31	0.29
Error	0.00	23	0.00		
3) TIME	0.00	1	0.00	0.33	0.57
TIME*Mix + rate	0.00	5	0.00	0.20	0.96
TIME*Soil type	0.00	1	0.00	20.37	***0.00***
TIME*Mix + rate * Soil type	0.00	5	0.00	0.33	0.89
Error	0.00	23	0.00		

Table A.1-5 Analysis of variance for total soil phosphorous at the start and end of incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	0.06	5	0.0	0.5	0.78
2) Soil type	0.50	1	0.5	22.3	***0.00***
Mix + rate*Soil type	0.09	5	0.0	0.8	0.57
Error	0.54	24	0.0		
3) TIME	0.02	1	0.0	1.0	0.33
TIME*Mix + rate	0.12	5	0.0	1.2	0.32
TIME*Soil type	0.92	1	0.9	48.6	***0.00***
TIME*Mix + rate*Soil type	0.05	5	0.0	0.5	0.74
Error	0.46	24	0.0		

Table A.1-6 Analysis of variance for Ammonium for soil samples collected at the start and day 30.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	429.4	5	85.88	155.7	***0.00***
2) Soil type	0.4	1	0.40	0.7	0.41
Mix + rate*Soil type	16.4	5	3.28	5.9	0.00
Error	12.7	23	0.55		
3) TIME	583.3	1	583.33	1215.7	***0.00***
TIME*Mix + rate	414.4	5	82.88	172.7	***0.00***
TIME*Soil type	0.0	1	0.03	0.1	0.81
TIME*Mix + rate*Soil type	4.5	5	0.91	1.9	0.13
Error	11.0	23	0.48		

Table A.1-7 Analysis of variance for soil microbial biomass nitrogen for soil samples collected at during the soil incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Mix + rate	2133	5	427	0.93	0.48
2) Soil type	76	1	76	0.17	0.69
Mix + rate*Soil type	960	5	192	0.42	0.83
Error	9198	20	460		
3) TIME	9234	4	2308	5.69	***0.00***
TIME*Mix + rate	8306	20	415	1.02	0.44
TIME*Soil type	2210	4	552	1.36	0.25
TIME*Mix + rate*Soil type	14774	20	739	1.82	***0.03***
Error	32430	80	405		

Table A.1-8 Analysis of variance for soil microbial biomass carbon for soil samples collected at during the soil incubation experiment.

Source of variation	SS	D.o.F	MS	F	p
Mix + rate	12335	5	2467	3.9	***0.03***
Soil type	62003	1	62003	99.0	***0.00***
Mix + rate*Soil type	5207	5	1041	1.7	0.22
Error	6888	11	626		
Time	13239	4	3310	2.7	***0.04***
Time*Mix + rate	92365	20	4618	3.8	***0.00***
Time*Soil type	1816	4	454	0.4	0.83
Time*Mix + rate*Soil type	41456	20	2073	1.7	0.07
Error	53782	44	1222		

Appendix B Chapter 4

B.1 Experimental monitoring

B.1.1 Experimental activities

Table B.1-1 presences a list of major activities carried out during the pot experiment. The major activities included sewage effluent collection and irrigation and cutting of ryegrass.

Table B.1-1 Dates of experimental activities and monitoring

Date	Activity
10/04/2010	Packing soil (6.3 kg) into 5 L pots.
12/04/2010	Determination of field capacity
20/04/2010	Compost application, planting of ryegrass seeds
21/04/2010	Day zero soil samples collected
30/04/2010	Start of germination of ryegrass
18/05/2010	STSE starts
25/05/2010	Planting of additional seeds in all pots (1 g).
15/06/2010	Weights of pot were taken to check the amount of water in the pots
25/06/2010	First ryegrass cut and soil sampling done.
28/07/2010	Spraying with cypermethrin
06/08/2010	Second grass cut and second sampling
01/02/2011	Third ryegrass grass cut done. End of year 1 and start of year 2
25/04/2011	First ryegrass cut of the second year.
09/06/2011	Second ryegrass cut of the second year
26/07/2011	Third ryegrass cut
16/11/2011	Fourth ryegrass cut
27/04/2012	Fifth (last) ryegrass cut
30/04/2012	Soil sampling

B.1.2 STSE irrigation

Table B.1-2 Treated effluent irrigation to supply 75 kg N ha^{-1} in the first year (2010/11)

Month	Week	Irrigation (ml/week)	Combinations of compost and STSE (%)				
			$100_{compost}+0_{effluent}$	$75_{compost}+25_{effluent}$	$50_{compost}+50_{effluent}$	$25_{compost}+75_{effluent}$	$0_{compost}+100_{effluent}$
April	1	720					
May	2	510			**Deionised water**		
	3	690					
	4	674		**Effluent**	**Effluent**	**Effluent**	
	5	208					
June	6	0	**Deionised water**				**Effluent**
	7	0					
	8	499					
	9	520		**1730**			
July	10	1129					
	11	1860			**3413**		
	12	1395				**5125**	
	13	1661		**Deionised water**			
Aug	14	1130					**6817**
Total irrigation		10996					

Table B.1-3 Treated effluent irrigation to supply 150 kg N ha^{-1} in the first year (2010/11)

Month	Week	Irrigation (ml/week)	Combinations of compost and STSE (%)				
			$100_{compost}+0_{effluent}$	$75_{compost}+25_{effluent}$	$50_{compost}+50_{effluent}$	$25_{compost}+75_{effluent}$	$0_{compost}+100_{effluent}$
April	1	720					
May	2	510			Deionised water		
	3	690					
	4	674		Effluent	Effluent	Effluent	
	5	208					
June	6	0					Effluent
	7	0					
	8	499					
	9	520					
July	10	1129					
	11	1860		3493			
	12	1395	Deionised				
	13	1661	water		6767		
Aug	14	1130					
	15	1063					
	16	931		Deionised		9873	
	17	797		water	Deionised		
	18	1070			water	Deionised	
Sept	19	997				water	
	20	465					12972
Total irrigation		16318					

Table B.1-4 Treated effluent irrigation to supply 75 kg N ha^{-1} in the second year (2011/12)

			Combinations of compost and STSE (%)				
Month	**Week**	**Irrigation (ml/pot/week)**	$100_{compost}+0_{effluent}$	$75_{compost}+25_{effluent}$	$50_{compost}+50_{effluent}$	$25_{compost}+75_{effluent}$	$0_{compost}+100_{effluent}$
February	1	0					
	2	432					
	3	465					
	4	0					
March	5	465					
	6	0					
	7	0					
	8	532		**1894**			
April	9	465					
	10	0					
	11	332					
	12	864	**Deionised**		**3366**		
May	13	465	**water**				
	14	1130		**Deionised**		**5149**	
	15	997		**water**			
	16	930					**7076**
June	17	0			**Deionised**	**Deionised**	
	18	0			**water**	**water**	**Deionised**
	19						**water**
Total irrigation		7076					

Table B.1-5 Treated effluent irrigation to supply 150 kg N ha^{-1} in the second year (2011/12)

			Combinations of compost and STSE (%)				
Month	**Week**	**Irrigation (ml/pot/week)**	$100_{compost}+0_{effluent}$	$75_{compost}+25_{effluent}$	$50_{compost}+50_{effluent}$	$25_{compost}+75_{effluent}$	$0_{compost}+100_{effluent}$
February	1	0					
	2	432					
	3	465					
	4	0					
March	5	465					
	8	532					
April	9	465					
	10	0					
	11	332					
	12	864		**3385**			
May	13	465					
	14	1130					
	15	997					
	16	930			**7076**		
June	17	930					
	18	399					
	19	399					
	20	0		Deionised water			
July	21	532					
	22	0					
	23	399					
	24	0					
August	25	1063				**10797**	
	26	997					
	27	930					
	28	532					
Sept	29	399					13654
Total irrigation		13654	13654				

B.1.3 STSE analysis

Table B.1-6 Sewage Effluent analysis for the first year (2010/2011) of the pot experiment

Analysis	Sample date	Sample 1 (mg l^{-1})	Sample 2 (mg l^{-1})	Sample 3 (mg l^{-1})
Total Dissolved N	18/05/2010	34	42	42
	21/05/2010	38	33	39
	25/05/2010	28	28	28
	15/06/2010	28	27	29
	08/07/2010	35	33	34
	15/07/2010	39	36	38
	22/07/2010	33	34	35
	29/07/2010	43	46	48
	17/08/2010	43	34	37
Ammonium	18-May-10	7.02	7.03	6.94
	21-May-10	6.93	6.84	6.81
	26-May-10	7.65	7.6	7.57
	15-Jun-10	7.5	7.47	7.46
	03/07/2010	6.59	6.63	6.64
	15/07/2010	6.12	6.43	6.62
	22/07/2010	6.69	6.45	6.52
	29/07/2010	6.14	6.16	6.2
	17/08/2010	6.31	6.09	6.19
Nitrate	18-May-10	27.7	25.4	25.7
	21-May-10	33.6	32.8	34.3
	25-May-10	24.1	22.5	22.9
	15-Jun-10	22	20.7	22.3
	03/07/2010	28.8	27.8	27.7
	05/05/2010	32.5	28.2	29.4
	22/07/2010	24.3	24.4	23.9
	29/07/2010	31.9	31.1	31.2
	17/08/2010	27.5	28.9	29
Dissolved Total P	18-May-10	6.4	6.4	6.4
	15-Jun-10	5	6.6	4.8
	08-Jul-10	6	6.2	6
	17/08/2010	4.8	4.9	4.9
Dissolved K	18-May-10	32.1	20.5	21.4
	15-Jun-10	21.9	20.9	19.5
	08-Jul-10	24.5	24.5	23
	17/08/2010	18.1	18.5	19.1

Table B.1-7 Sewage Effluent analysis for the Second year (2011/2012) of the pot experiment

Analysis	Sample date	Sample 1 (mg l^{-1})	Sample 2 (mg l^{-1})	Sample 3 (mg l^{-1})
Dissolved total N	07/02/2011	49	52	53
	18/02/2011	48	49	48
	08/03/2011	44	43	45
	25/03/2011	50	48	52
	07/04/2011	60	54	57
	06/05/2011	73	53	51
	26/05/2011	71	76	67
	31/05/2011	65	58	56
Ammonium	07-Feb-11	2.6	2.6	2.6
	18-Feb-11	3.6	3.5	3.6
	08-Mar-11	0.4	0.3	0.3
	25/03/2011	5.1	5.4	5.8
	07/04/2011	1.5	1.5	1.5
	06/05/2011	5.1	4.9	4
	26/05/2011	8	7.7	7.3
	31/05/2011	3.5	3.2	3.4
	18/07/2011	1.3	1.3	1.3
	02/08/2011	0.7	0.4	0.2
	05/08/2011	0.7	0.7	0.3
	24/08/2011	0.8	0.6	0.6
Nitrate	07-Feb-11	38.4	39	39.1
	18-Feb-11	43.1	42.8	43.1
	08-Mar-11	42.2	41.4	42.2
	25/3/11	32.6	35.4	29.2
	07/04/2011	49.2	49.5	50.2
	06/05/2011	41.8	42.2	41.5
	26/05/2011	49.4	46	42.7
	31/05/2011	51.4	50.9	51.1
	18/07/2011	33.2	34	35.1
	02/08/2011	35.4	32.8	33.3
	05/08/2011	34.4	35	33.7
	24/08/2011	36.1	34.9	35.4
Total P	07-Feb-11	8.2	8.4	8.3
	08-Mar-11	6.6	6.7	6.6
	07/04/2011	6.9	7	6.9
	06/05/2011	6	5.9	5.9
	18/07/2011	6.6	6.5	6.5
	24/08/2011	6.2	6.2	6.3

Analysis	Sample date	Sample 1 (mg l^{-1})	Sample 2 (mg l^{-1})	Sample 3 (mg l^{-1})
Total K	07-Feb-11	17.6	16.6	17.8
	08-Mar-11	18.2	18.7	18.4
	07/04/2011	29.2	25.2	26.3
	06/05/2011	25.1	24.4	24.3
	18/07/2011	18	26	23
	22/07/2011	22.8	21.2	22
pH	07-Feb-11	6.42	6.37	6.31
	18-Feb-11	6.03	6.2	6.13
	08-Mar-11	6.09	6.03	6.13
	25/03/2011	6.27	6.25	6.18
	07/04/2011	6.24	6.29	6.3
	06/05/2011	5.83	5.78	5.62
	26/05/2011	6.08	6.2	6.25
	31/05/2011	6.5	6.1	6.03
	18/07/2011	7.52	7.36	7.4
	02/08/2011	7.86	7.74	7.82
	05/08/2011	7.89	7.91	7.87
	24/08/2011	7.58	7.65	7.74
Conductivity	07-Feb-11	770	778	783
	18-Feb-11	826	806	830
	08-Mar-11	781	785	784
	25/03/2011	801	808	800
	07/04/2011	1032	1046	1048
	06/05/2011	802	799	796
	26/05/2011	855	857	861
	31/05/2011	847	810	818
	18/07/2011	844	851	854
	02/08/2011	951	978	944
	05/08/2011	868	844	927
	24/08/2011	878	867	844

B.2 Statistics

B.2.1 Statistical analyses

Table B.2-1 Analysis of variance for total dry matter for year 1 (2010/11) and year 2 (20l1/12) for the glasshouse experiment.

Source	SS	D.o.F	MS	F	p
(1) Compost/effluent combination (%)	107800459	4	26950115	297.6	***0.00***
(2) Application rate (kg N/ha)	35074642	1	35074642	387.3	***0.00***
(3) Soil type	201368788	1	201368788	2223.8	***0.00***
Compost/effluent combination (%)*Application rate (kg N/ha)	14766865	4	3691716	40.8	***0.00***
Compost/effluent combination (%)*Soil type	2596442	4	649110	7.2	***0.00***
Application rate (kg N/ha)*Soil type	580876	1	580876	6.4	***0.02***
Compost/effluent combination (%)*Application rate (kg N/ha)*Soil type	1028999	4	257250	2.8	***0.04***
Error	3259852	36	90551		
4) Time	84017357	1	84017357	843.3	***0.00***
Time*Compost/effluent nutrient combination (%)	9723943	4	2430986	24.4	***0.00***
Time*Application rate (kg N ha^{-1})	2552542	1	2552542	25.6	***0.00***
Time*Soil type	93760717	1	93760717	941.1	***0.00***
Time*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	3518245	4	879561	8.8	***0.00***
Time*Compost/effluent Nutrient combination (%)*Soil type	346954	4	86738	0.9	0.49
Time*Application rate (kg N ha^{-1})*Soil type	35991	1	35991	0.4	0.55
4*1*2*3	977302	4	244325	2.5	0.06
Error	3586728	36	99631		

Table B.2-2 Analysis of variance for total dry matter for year 1 (2010/11)

Source	SS	D.o.F	MS	F	p
(1) Compost/effluent combination (%)	12555845	4	3138961	70.1	***0.00***
(2) Application rate (kg N/ha)	3583586	1	3583586	80.0	***0.00***
3) Soil type	97806663	1	97806663	2183	***0.00***
Compost/effluent combination (%)*Application rate (kg N/ha)	2014015	4	503504	11.2	***0.00***
Compost/effluent combination (%)*Soil type	458394	4	114598	2.6	0.05
Application rate (kg N/ha)*Soil type	83620	1	83620	1.9	0.18
Compost/effluent combination (%)*Application rate (kg N/ha)*Soil type	480040	4	120010	2.7	***0.05***
Error	1702373	38	44799		
(4) Time	10695992	2	5347996	91	***0.00***
Time*Compost/effluent Nutrient combination (%)	10806285	8	1350786	23	***0.00***
Time*Application rate (kg N ha^{-1})	3559450	2	1779725	30	***0.00***
Time*Soil type	2205771	2	1102885	19	***0.00***
Time*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	5387014	8	673377	11	***0.00***
Time*Compost/effluent Nutrient combination (%)*Soil type	762362	8	95295	1.6	0.13
Time*Application rate (kg N ha^{-1})*Soil type	53641	2	26820	0.5	0.63
4*1*2*3	520909	8	65114	1.1	0.37
Error	4455799	76	58629		

Table B.2-3 Analysis of variance for total dry matter for year 2 (2011/12)

Source of variation	SS	D.o.F	MS	F	p
(1) Compost/effluent combination (%)	16743577	4	4185894	255.6	***0.00***
(2) Application rate (kg N/ha)	5655119	1	5655119	345.4	***0.00***
(3) Soil type	2031697	1	2031697	124.1	***0.00***
Compost/effluent combination (%)*Application rate (kg N/ha)	2323080	4	580770	35.5	***0.00***
Compost/effluent combination (%)*Soil type	359468	4	89867	5.5	***0.00***
Application rate (kg N/ha)*Soil type	32769	1	32769	2.0	0.17
Compost/effluent combination (%)*Application rate (kg N/ha)*Soil type	189568	4	47392	2.9	***0.04***
Error	589453	36	16374		
4) Time	10666927	4	2666732	172.2	***0.00***
Time*Compost/effluent Nutrient combination (%)	9631761	16	601985	38.9	***0.00***
Time*Application rate (kg N ha^{-1})	1886964	4	471741	30.5	***0.00***
Time*Soil type	728147	4	182037	11.8	***0.00***
Time*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	5488012	16	343001	22.1	***0.00***
Time*Compost/effluent Nutrient combination (%)*Soil type	184682	16	11543	0.7	0.74
Time*Application rate (kg N ha^{-1})*Soil type	71171	4	17793	1.1	0.34
4*1*2*3	141715	16	8857	0.6	0.90
Error	2230282	144	15488		

Table B.2-4 Analysis of variance for total dry matter at the end of the 2 year study

Source of variation	SS	D.o.F	MS	F	p
Compost/effluent combination (%)	215600918	4	53900230	298	***0.00***
Application rate (kg N/ha)	70149284	1	70149284	387	***0.00***
Soil type	402737576	1	402737576	2224	***0.00***
Compost/effluent combination (%)*Application rate (kg N/ha)	29533729	4	7383432	41	***0.00***
Compost/effluent combination (%)*Soil type	5192883	4	1298221	7	***0.00***
Application rate (kg N/ha)*Soil type	1161752	1	1161752	6	***0.02***
Compost/effluent combination (%)*Application rate (kg N/ha)*Soil type	2057998	4	514500	3	***0.04***
Error	6519704	36	181103		

Table B.2-5 Analysis of variance for nitrogen use efficiency between year 1 and 2 of the glasshouse/pot experiment

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
1) Compost/effluent combinations (%)	8187	4	2047	16.9	***0.00***
2) Application rate (kg N ha^{-1})	26088	1	26088	2152	***0.00***
3) Soil type	22095	1	22095	1823	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	99	4	25	2	0.11
Compost/effluent Nutrient combination (%)*Soil type	301	4	75	6	***0.00***
Application rate (kg N ha^{-1})*Soil type	2996	1	2996	247	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	93	4	23	2	0.13
Error	461	38	12		
4) Year	9889	1	9889	1086	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	718	4	180	20	***0.00***
YEAR*Application rate (kg N ha-1)	2060	1	2060	226	***0.00***
YEAR*Soil type	9898	1	9898	1087	***0.00***
YEAR*Compost/effluent nutrient combination (%)*Application rate (kg N ha^{-1})	151	4	38	4	***0.01***
YEAR*Compost/effluent Nutrient combination (%)*Soil type	22	4	5	1	0.67
YEAR*Application rate (kg N ha^{-1})*Soil type	1101	1	1101	121	***0.00***
4 x 3 x 2 x 1	42	4	10	1	0.35
Error	346	38	9		

Table B.2-6 Analysis of variance total N uptake for first and second year of the pot experiment

Source of variation	SS	D.o.F	MS	F	p
(1) Compost/effluent combinations (%)	49141	4	12285	177.4	***0.00***
(2) Application rate (kg N ha^{-1})	18349	1	18349	264.9	***0.00***
(3) Soil type	148232	1	148232	2139.9	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	9589	4	2397	34.6	***0.00***
Compost/effluent Nutrient combination (%)*Soil type	99	4	25	0.4	0.84
Application rate (kg N ha^{-1})*Soil type	1	1	1	0.0	0.92
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	329	4	82	1.2	0.33
Error	2632	38	69		
4) Year	61247	1	61247	974.2	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	2625	4	656	10.4	***0.00***
YEAR*Application rate (kg N ha-1)	1970	1	1970	31.3	***0.00***
YEAR*Soil type	112213	1	112213	1784.8	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	2156	4	539	8.6	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Soil type	958	4	239	3.8	***0.01***
YEAR*Application rate (kg N ha^{-1})*Soil type	64	1	64	1.0	0.32
4 x 3 x 3 x 1	1094	4	274	4.4	***0.01***
Error	2389	38	63		

Table B.2-7 Analysis of variance for total N uptake for ryegrass cuts made in the first year (2010/11) of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (kg N ha^{-1})	5841	4	1460	51	***0.00***
2) Application rate (%)	1360	1	1360	47	***0.00***
3) Soil type	84468	1	84468	2933	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	1187	4	297	10.3	***0.00***
Compost/effluent Nutrient combination (%)*Soil type	178	4	44	1.5	0.21
Application rate (kg N ha^{-1})*Soil type	9	1	9	0.3	0.58
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	286	4	71	2.5	0.06
Error	1066	37	29		
4) Year	56626	2	28313	1005	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	5341	8	668	24	***0.00***
YEAR*Application rate (kg N ha^{-1})	1664	2	832	30	***0.00***
YEAR*Soil type	25926	2	12963	460	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	861	8	108	4	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Soil type	280	8	35	1.2	0.29
YEAR*Application rate (kg N ha^{-1})*Soil type	4	2	2	0.1	0.92
4 x 3 x 2 x 1	184	8	23	0.8	0.59
Error	2084	74	28		

Table B.2-8 Analysis of variance for total N uptake for ryegrass cuts made in the second (2011/12) of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations	6517	4	1629	166	***0.00***
2) Application rate	3142	1	3142	320	***0.00***
3) Soil type	240	1	240	24	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	1578	4	395	40	***0.00***
Compost/effluent Nutrient combination (%)*Soil type	95	4	24	2	0.07
Application rate (kg N ha^{-1})*Soil type	8	1	8	1	0.36
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	107	4	27	3	***0.04***
Error	363	37	10		
4) Year	9307	4	2327	409	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	6037	16	377	66	***0.00***
YEAR*Application rate (kg N ha^{-1})	1672	4	418	74	***0.00***
YEAR*Soil type	62	4	16	3	***0.03***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	3274	16	205	36	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Soil type	242	16	15	3	***0.00***
YEAR*Application rate (kg N ha^{-1})*Soil type	26	4	7	1	0.34
4 x 3 x 3 x 1	176	16	11	2	***0.02***
Error	842	148	6		

Table B.2-9 Anaysis of variance for soil carbon for soil samples at the end of first and second year of the pot experiment

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations	0.04	4	0.01	1.70	0.17
2) Application rate	0.05	1	0.05	8.30	***0.01***
3) Soil type	17.4	1	17.4	2946	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)	0.01	4	0.00	0.34	0.85
Compost/effluent Nutrient combination (%)*Soil type	0.03	4	0.01	1.47	0.23
Application rate (kg N ha-1)*Soil type	0.00	1	0.00	0.04	0.84
Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)*Soil type	0.05	4	0.01	2.18	0.09
Error	0.23	39	0.01		
4) Year	0.25	1	0.25	69.12	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	0.01	4	0.00	0.66	0.62
YEAR*Application rate (kg N ha-1)	0.00	1	0.00	0.50	0.48
YEAR*Soil type	0.02	1	0.02	5.10	***0.03***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)	0.02	4	0.01	1.43	0.24
YEAR*Compost/effluent Nutrient combination (%)*Soil type	0.03	4	0.01	1.75	0.16
YEAR*Application rate (kg N ha-1)*Soil type	0.02	1	0.02	5.80	***0.02***
4 x 3 x 3 x 1	0.04	4	0.01	3.08	***0.03***
Error	0.14	39	0.00		

Table B.2-10 Analysis of variance for soil organic matter for soil samples at the end of first and second year of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
(1) Compost/effluent Nutrient combination (%)	0.03	4	0.01	0.31	0.871
(2) Application rate (kg N ha^{-1})	0.17	1	0.17	6.29	***0.016***
(3) Soil type	60	1	60.43	2286	***0.000***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.08	4	0.02	0.76	0.560
Compost/effluent Nutrient combination (%)*Soil type	0.08	4	0.02	0.79	0.538
Application rate (kg N ha^{-1})*Soil type	0.02	1	0.02	0.57	0.455
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	0.14	4	0.04	1.33	0.275
Error	1.06	40	0.03		
(4) Time	0.22	1	0.22	4.70	***0.036***
Time*Compost/effluent Nutrient combination (%)	0.10	4	0.03	0.55	0.699
Time*Application rate (kg N ha^{-1})	0.01	1	0.01	0.23	0.632
Time*Soil type	0.54	1	0.54	11.84	***0.001***
Time*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.28	4	0.07	1.51	0.217
Time*Compost/effluent Nutrient combination (%)*Soil type	0.06	4	0.02	0.35	0.846
Time*Application rate (kg N ha^{-1})*Soil type	0.05	1	0.05	1.04	0.313
4*1*2*3	0.02	4	0.00	0.11	0.980
Error	1.84	40	0.05		

Table B.2-11 Analysis of variance for total N in soil for soil samples at the end of first and second year of the pot experiment.

Source	SS	D.o.F	MS	F	p
1) Compost/effluent combinations	0.0004	4	0.0001	2.1	0.10
2) Application rate	0.0001	1	0.0001	2.7	0.11
3) Soil type	0.1293	1	0.1293	3023	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.0001	4	0.0000	0.3	0.86
Compost/effluent Nutrient combination (%)*Soil type	0.0001	4	0.0000	0.8	0.55
Application rate (kg N ha^{-1})*Soil type	0.0000	1	0.0000	0.2	0.65
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	0.0003	4	0.0001	1.8	0.14
Error	0.0017	39	0.0000		
4) Year	0.0123	1	0.0123	659.3	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	0.0002	4	0.0000	2.1	0.11
YEAR*Application rate (kg N ha^{-1})	0.0001	1	0.0001	3.3	0.08
YEAR*Soil type	0.0001	1	0.0001	5.7	***0.02***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.0002	4	0.0000	2.6	0.05
YEAR*Compost/effluent Nutrient combination (%)*Soil type	0.0002	4	0.0001	3.0	***0.03***
YEAR*Application rate (kg N ha^{-1})*Soil type	0.0001	1	0.0001	6.7	***0.01***
4 x 3 x 2 x 1	0.0002	4	0.0001	3.2	***0.02***
Error	0.0007	39	0.0000		

Table B.2-12 Analysis of variance for total P for soil samples at the end of first and second year of the pot experiment.

Source	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	0.02	4	0.00	0.27	0.90
2) Application rate (kg N ha^{-1})	0.00	1	0.00	0.01	0.92
3) Soil type	3.21	1	3.21	213.20	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.02	4	0.00	0.31	0.87
Compost/effluent Nutrient combination (%)*Soil type	0.02	4	0.01	0.38	0.82
Application rate (kg N ha^{-1})*Soil type	0.00	1	0.00	0.13	0.72
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	0.02	4	0.00	0.25	0.91
Error	0.57	38	0.02		
4) Year	0.34	1	0.34	36.48	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	0.00	4	0.00	0.11	0.98
YEAR*Application rate (kg N ha^{-1})	0.04	1	0.04	3.77	0.06
YEAR*Soil type	0.01	1	0.01	1.42	0.24
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.02	4	0.00	0.47	0.76
YEAR*Compost/effluent Nutrient combination (%)*Soil type	0.04	4	0.01	1.05	0.39
YEAR*Application rate (kg N ha^{-1})*Soil type	0.00	1	0.00	0.47	0.50
4 x 3 x 3 x 1	0.05	4	0.01	1.28	0.29
Error	0.36	38	0.01		

Table B.2-13 Analysis of variance for soil mineral N in the soil at the start and end of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	133	4	33	0.9	0.45
2) Application rate (kg N ha^{-1})	31	1	31	0.9	0.35
3) Soil type	387	1	387	11.0	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	73	4	18	0.5	0.72
Compost/effluent Nutrient combination (%)*Soil type	244	4	61	1.7	0.16
Application rate (kg N ha^{-1})*Soil type	6	1	6	0.2	0.67
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	158	4	39	1.1	0.36
Error	1300	37	35		
4) Year	4396	1	4396	127.2	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	123	4	31	0.9	0.48
YEAR*Application rate (kg N ha^{-1})	29	1	29	0.8	0.37
YEAR*Soil type	438	1	438	12.7	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	75	4	19	0.5	0.70
YEAR*Compost/effluent Nutrient combination (%)*Soil type	208	4	52	1.5	0.22
YEAR*Application rate (kg N ha^{-1})*Soil type	7	1	7	0.2	0.65
4 x 3 x 3 x 1	148	4	37	1.1	0.39
Error	1279	37	35		

Table B.2-14 Analysis of variance for Copper in the soil at the start and end of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	12	4	3.0	0.4	0.80
2) Application rate (kg N ha^{-1})	1	1	1.4	0.2	0.66
3) Soil type	87	1	87.5	12.3	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	4	4	0.9	0.1	0.97
Compost/effluent Nutrient combination (%)*Soil type	22	4	5.5	0.8	0.55
Application rate (kg N ha^{-1})*Soil type	5	1	5.3	0.8	0.39
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	19	4	4.9	0.7	0.61
Error	276	39	7.1		
4) Year	456	1	455.5	80.5	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	4	4	0.9	0.2	0.96
YEAR*Application rate (kg N ha^{-1})	0	1	0.1	0.0	0.92
YEAR*Soil type	1	1	0.8	0.1	0.71
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	15	4	3.8	0.7	0.62
YEAR*Compost/effluent Nutrient combination (%)*Soil type	52	4	13.1	2.3	0.07
YEAR*Application rate (kg N ha^{-1})*Soil type	11	1	10.7	1.9	0.18
4 x 3 x 3 x 1	11	4	2.6	0.5	0.76
Error	221	39	5.7		

Table B.2-15 Analysis of variance for Lead in the soil at the start and end of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	5837	4	1459	1.5	0.23
2) Application rate (kg N ha^{-1})	438	1	438	0.4	0.51
3) Soil type	30900	1	30900	31.4	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	4642	4	1161	1.2	0.34
Compost/effluent Nutrient combination (%)*Soil type	4394	4	1098	1.1	0.36
Application rate (kg N ha^{-1})*Soil type	1595	1	1595	1.6	0.21
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	4448	4	1112	1.1	0.36
Error	35431	36	984		
4) Year	12458	1	12458	32.0	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	2485	4	621	1.6	0.20
YEAR*Application rate (kg N ha^{-1})	489	1	489	1.3	0.27
YEAR*Soil type	10254	1	10254	26.3	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	3254	4	813	2.1	0.10
YEAR*Compost/effluent Nutrient combination (%)*Soil type	2083	4	521	1.3	0.27
YEAR*Application rate (kg N ha^{-1})*Soil type	931	1	931	2.4	0.13
4 x 3 x 2 x 1	4799	4	1200	3.1	***0.03***
Error	14011	36	389		

Table B.2-16 Analysis of variance for Nickel in the soil at the start and end of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	151	4	38	0.50	0.73
2) Application rate (kg N ha^{-1})	106	1	106	1.42	0.24
3) Soil type	0	1	0	0.01	0.94
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	233	4	58	0.77	0.55
Compost/effluent Nutrient combination (%)*Soil type	46	4	11	0.15	0.96
Application rate (kg N ha^{-1})*Soil type	5	1	5	0.06	0.80
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	139	4	35	0.46	0.76
Error	2330	31	75		
4) Year	30	1	30	1.49	0.23
YEAR*Compost/effluent Nutrient combination (%)	73	4	18	0.91	0.47
YEAR*Application rate (kg N ha^{-1})	196	1	196	9.77	***0.00***
YEAR*Soil type	14	1	14	0.69	0.41
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	67	4	17	0.84	0.51
YEAR*Compost/effluent Nutrient combination (%)*Soil type	53	4	13	0.66	0.62
YEAR*Application rate (kg N ha^{-1})*Soil type	12	1	12	0.61	0.44
4 x 3 x 2 x 1	111	4	28	1.38	0.26
Error	620	31	20		

Table B.2-17 Analysis of variance for Zinc in the soil at the start and end of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	213	4	53.3	0.07	0.99
2) Application rate (kg N ha^{-1})	768	1	767.6	1.06	0.31
3) Soil type	5649	1	5648.6	7.78	***0.01***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	1946	4	486.6	0.67	0.62
Compost/effluent Nutrient combination (%)*Soil type	1928	4	481.9	0.66	0.62
Application rate (kg N ha^{-1})*Soil type	31	1	31.5	0.04	0.84
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	581	4	145.3	0.20	0.94
Error	23243	32	726.4		
4) Year	435	1	434.9	2.29	0.14
YEAR*Compost/effluent Nutrient combination (%)	279	4	69.7	0.37	0.83
YEAR*Application rate (kg N ha^{-1})	0	1	0.0	0.00	1.00
YEAR*Soil type	3	1	2.8	0.01	0.90
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	249	4	62.1	0.33	0.86
YEAR*Compost/effluent Nutrient combination (%)*Soil type	51	4	12.7	0.07	0.99
YEAR*Application rate (kg N ha^{-1})*Soil type	5	1	5.0	0.03	0.87
4 x 3 x 2 x 1	336	4	84.0	0.44	0.78
Error	6081	32	190.0		

Table B.2-18 Analysis of variance for Chromium in the soil at the start and end of the pot experiment.

Source	SS	D.o.F	MS	F	p
(1) Compost/effluent combinations (%)	191	4	48	0.83	0.52
(2) Application rate (kg N ha^{-1})	60	1	60	1.04	0.32
(3) Soil type	813	1	813	14.1	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*	100	4	25	0.43	0.78
Compost/effluent Nutrient combination (%)*Soil type	114	4	29	0.5	0.74
Application rate (kg N ha^{-1})*Soil type	89	1	89	1.55	0.22
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	303	4	76	1.32	0.28
Error	2191	38	58		
(4) Year	4790	1	4790	43.82	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	86	4	21	0.2	0.94
YEAR*Application rate (kg N ha^{-1})*	451	1	451	4.13	***0.05***
YEAR*Soil type	89	1	89	0.81	0.37
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*	302	4	75	0.69	0.6
YEAR*Compost/effluent Nutrient combination (%)*Soil type	154	4	38	0.35	0.84
YEAR*Application rate (kg N ha^{-1})*Soil type	7	1	7	0.07	0.8
4 x 3 x 2 x 1	112	4	28	0.26	0.9
Error	4154	38	109		

Table B.2-19 Analysis of variance for pH for soil samples at the end of first and second year of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	0.13	4	0.03	5	***0.00***
2) Application rate (kg N ha^{-1})	0.01	1	0.01	1	0.27
3) Soil type	2.21	1	2.21	345	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.03	4	0.01	1	0.31
Compost/effluent Nutrient combination (%)*Soil type	0.09	4	0.02	3	***0.02***
Application rate (kg N ha^{-1})*Soil type	0.10	1	0.10	15	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	0.23	4	0.06	9	***0.00***
Error	0.25	39	0.01		
4) Year	0.05	1	0.05	5	***0.03***
YEAR*Compost/effluent Nutrient combination (%)	0.03	4	0.01	1	0.62
YEAR*Application rate (kg N ha^{-1})	0.01	1	0.01	1	0.24
YEAR*Soil type	0.01	1	0.01	1	0.32
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	0.00	4	0.00	0	1.00
YEAR*Compost/effluent Nutrient combination (%)*Soil type	0.26	4	0.07	7	***0.00***
YEAR*Application rate (kg N ha^{-1})*Soil type	0.04	1	0.04	4	***0.04***
4 x 3 x 2 x 1	0.09	4	0.02	2	0.08
Error	0.39	39	0.01		

Table B.2-20 Analysis of variance for Extractable P for soil samples at the end of first and second year of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations (%)	24	4	6.1	0.9	0.45
2) Application rate	48	1	47.8	7.4	***0.01***
3) Soil type	5986	1	5985.9	930.3	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	39	4	9.7	1.5	0.22
Compost/effluent Nutrient combination (%)*Soil type	49	4	12.3	1.9	0.13
Application rate (kg N ha^{-1})*Soil type	2	1	1.8	0.3	0.60
Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})*Soil type	2	4	0.6	0.1	0.98
Error	251	39	6.4		
4) Year	1786	1	1785.6	297.6	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	14	4	3.4	0.6	0.68
YEAR*Application rate (kg N ha^{-1})	6	1	5.9	1.0	0.33
YEAR*Soil type	0	1	0.1	0.0	0.92
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha^{-1})	44	4	10.9	1.8	0.15
YEAR*Compost/effluent Nutrient combination (%)*Soil type	2	4	0.5	0.1	0.99
YEAR*Application rate (kg N ha^{-1})*Soil type	2	1	2.3	0.4	0.54
4 x 3 x 2 x 1	46	4	11.6	1.9	0.13
Error	234	39	6.0		

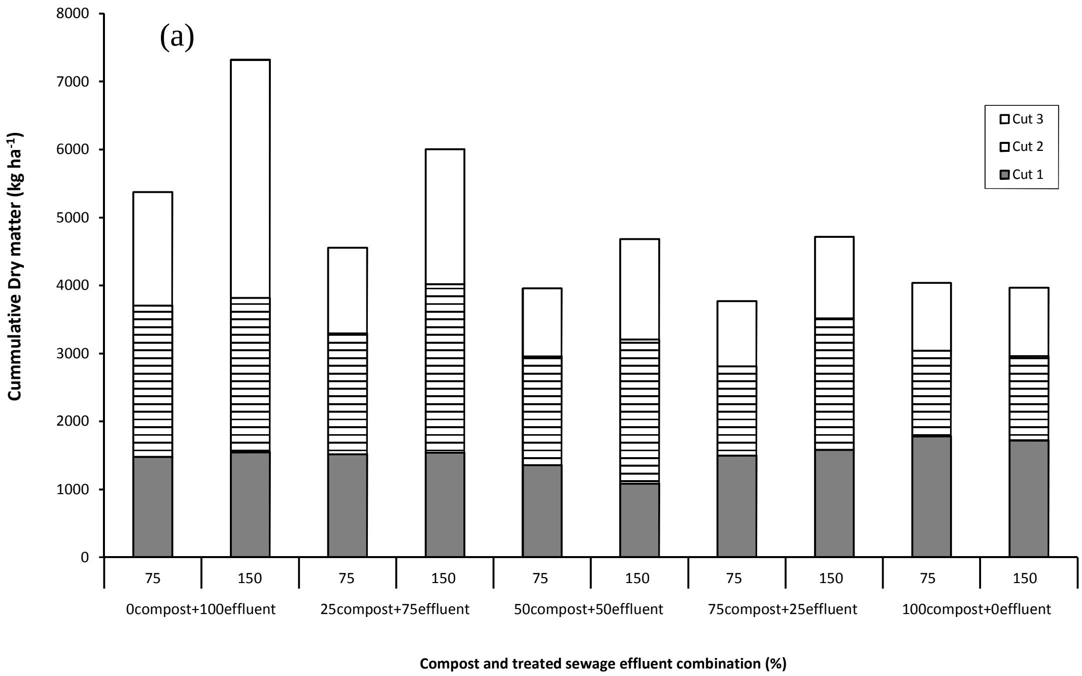
Table B.2-21 Analysis of variance for mean nitrogen content in plant material for first and second year of the pot experiment.

Source of variation	SS	D.o.F	MS	F	p
1) Compost/effluent combinations	0.69	4	0.17	15.3	***0.00***
2) Application rate	0.31	1	0.31	27.5	***0.00***
3) Soil type	4.34	1	4.34	383.6	***0.00***
Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)	0.21	4	0.05	4.7	***0.00***
Compost/effluent Nutrient combination (%)*Soil type	0.09	4	0.02	2.0	0.11
Application rate (kg N ha-1)*Soil type	0.01	1	0.01	0.9	0.34
Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)*Soil type	0.08	4	0.02	1.8	0.16
Error	0.44	39	0.01		
4) Year	1.45	1	1.45	152.0	***0.00***
YEAR*Compost/effluent Nutrient combination (%)	0.19	4	0.05	4.9	***0.00***
YEAR*Application rate (kg N ha-1)	0.00	1	0.00	0.0	0.91
YEAR*Soil type	5.92	1	5.92	621.0	***0.00***
YEAR*Compost/effluent Nutrient combination (%)*Application rate (kg N ha-1)	0.03	4	0.01	0.8	0.52
YEAR*Compost/effluent Nutrient combination (%)*Soil type	0.04	4	0.01	1.0	0.43
YEAR*Application rate (kg N ha-1)*Soil type	0.01	1	0.01	0.6	0.44
4 x 3 x 2 x 1	0.03	4	0.01	0.9	0.48
Error	0.37	39	0.01		

B.3 Glasshouse facility and ryegrass pictures

Figure B-1 The pot/glasshouse experiment in pictures; a) the glasshouse facility, b) pots prior to germination and c) ryegrass in pots.

B.4 Ryegrass dry matter



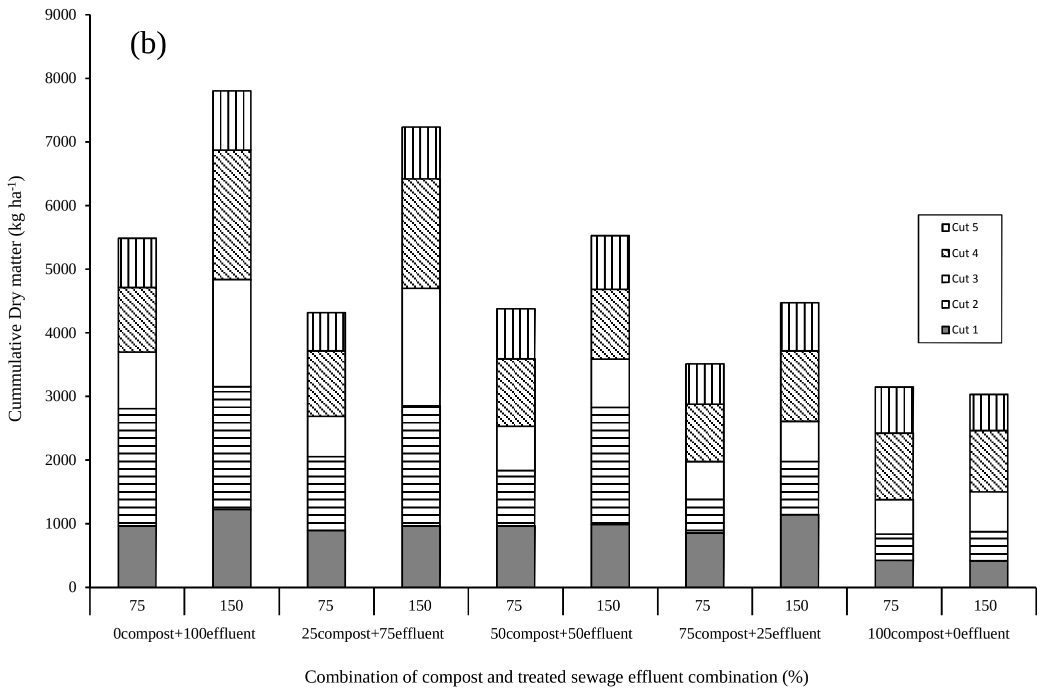
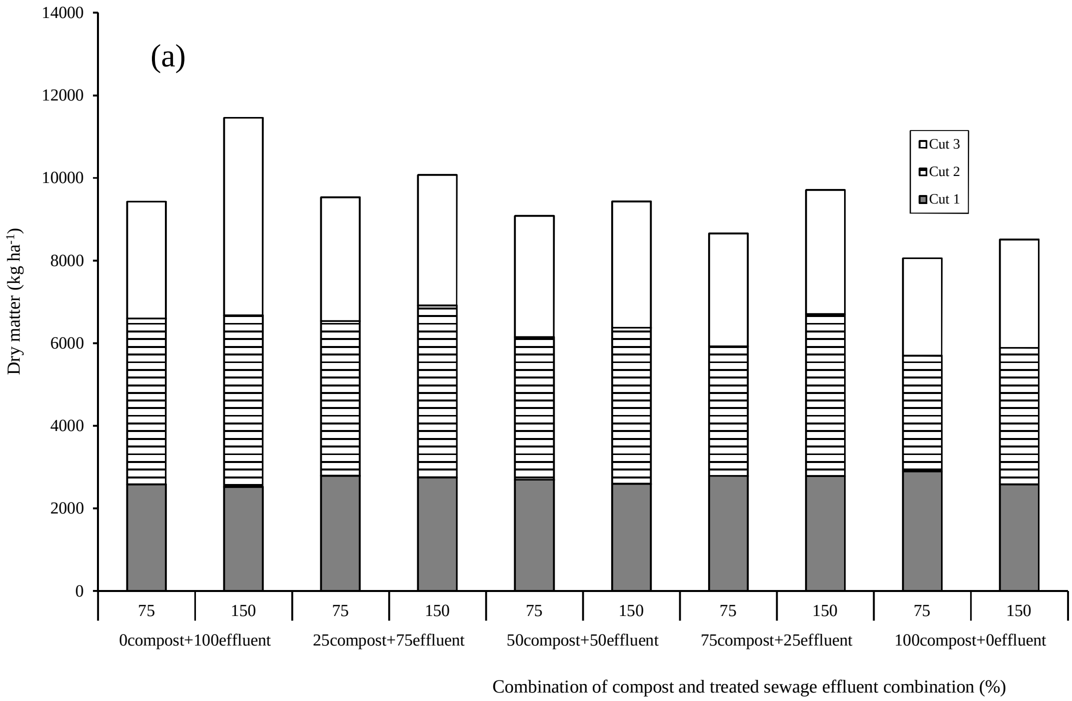


Figure B-2 Ryegrass dry matter harvested per cut in sandy loam in (a) first year (2010/11) and (b) 2011/12 in the pot experiment



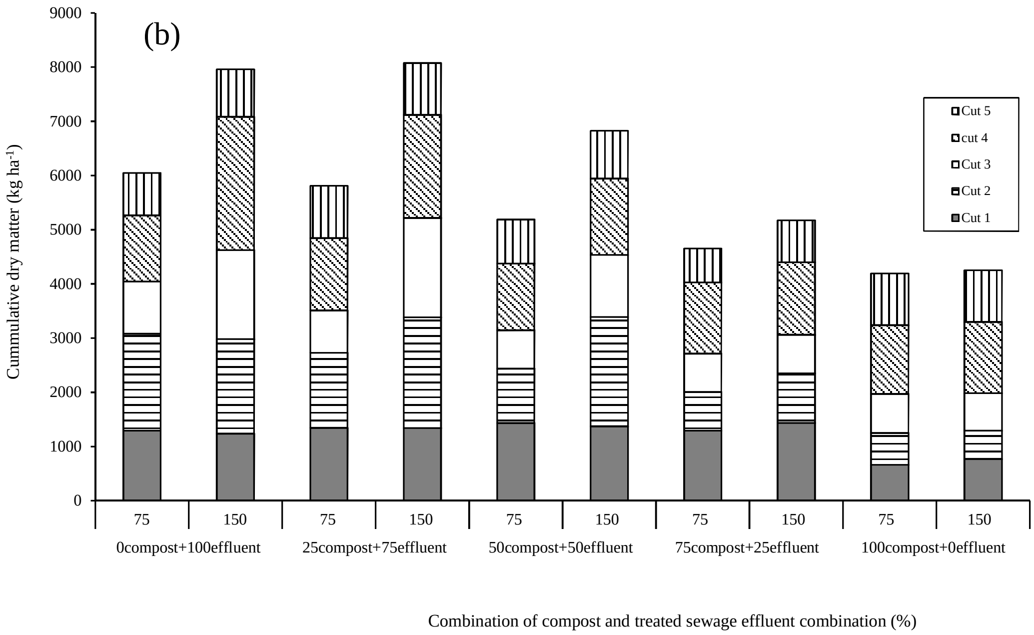


Figure B-3 Ryegrass dry matter harvested per cut in clay loam in (a) first year (2010/11) and (b) 2011/12 in the pot experiment

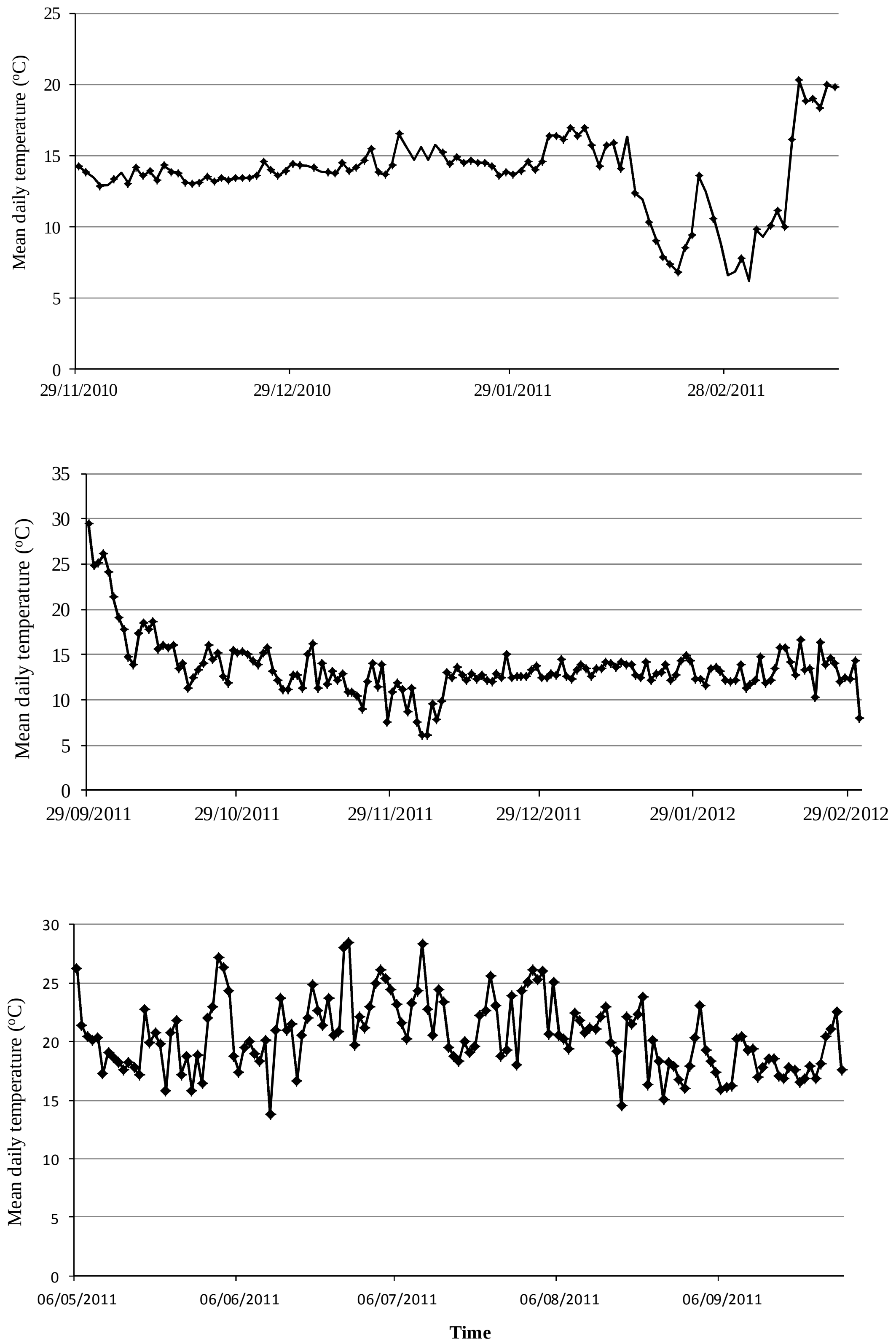


Figure B-4 Temperature records from the glasshouse (pot) experiment

Table B.4-1 Ryegrass DM (kg ha^{-1}) for the pot experiment in the first year (2010/11)

	($0_{compost}$+$100_{effluent}$)		**($25_{compost}$+$75_{effluent}$)**		**($50_{compost}$+$50_{effluent}$)**		**($75_{compost}$+$25_{effluent}$)**		**($100_{compost}$+$0_{effluent}$)**	
	N application rate (kg N ha^{-1})									
Clay loam	**75**	**150**	**75**	**150**	**75**	**150**	**75**	**150**	**75**	**150**
Cut 1	2582	2522	2791	2749	2693	2594	2786	2783	2898	2582
Cut 2	4017	4152	3747	4166	3454	3778	3139	3919	2802	3302
Cut 3	2828	4781	2996	3157	2936	3058	2729	3006	2357	2626
Sandy loam										
Cut 1	1480	1547	1515	1542	1358	1086	1498	1580	1780	1722
Cut 2	2227	2270	1780	2478	1601	2120	1316	1933	1260	1239
Cut 3	1670	3503	1261	1988	998	1475	955	1204	996	1008

Table B.4-2 Ryegrass DM (kg ha^{-1}) for the pot experiment in the second year (2011/12)

	($0_{compost}+100_{effluent}$)		($25_{compost}+75_{effluent}$)		($50_{compost}+50_{effluent}$)		($75_{compost}+25_{effluent}$)		($100_{compost}+0_{effluent}$)	
	N application rate (kg N ha^{-1})									
Clay loam	**75**	**150**	**75**	**150**	**75**	**150**	**75**	**150**	**75**	**150**
Cut 1	1296	1237	1343	1342	1434	1372	1294	1438	662	771
Cut 2	1784	1745	1387	2043	1003	2018	711	913	588	526
Cut 3	966	1643	782	1833	710	1145	709	711	722	689
Cut 4	1221	2458	1332	1901	1227	1411	1314	1338	1268	1316
Cut 5	781	875	966	955	817	882	628	772	955	950
Sandy loam										
Cut 1	964	1223	895	962	967	988	854	1142	422	415
Cut 2	1843	1930	1158	1889	865	1841	526	834	414	458
Cut 3	892	1684	633	1846	698	760	594	632	543	628
Cut 4	1011	2034	1030	1722	1062	1095	905	1106	1045	963
Cut 5	776	929	602	814	785	843	633	757	723	567

Appendix C Chapter 5

C.1 The lysimeter experiment

C.1.1 Layout and establishment of lysimeter experiment

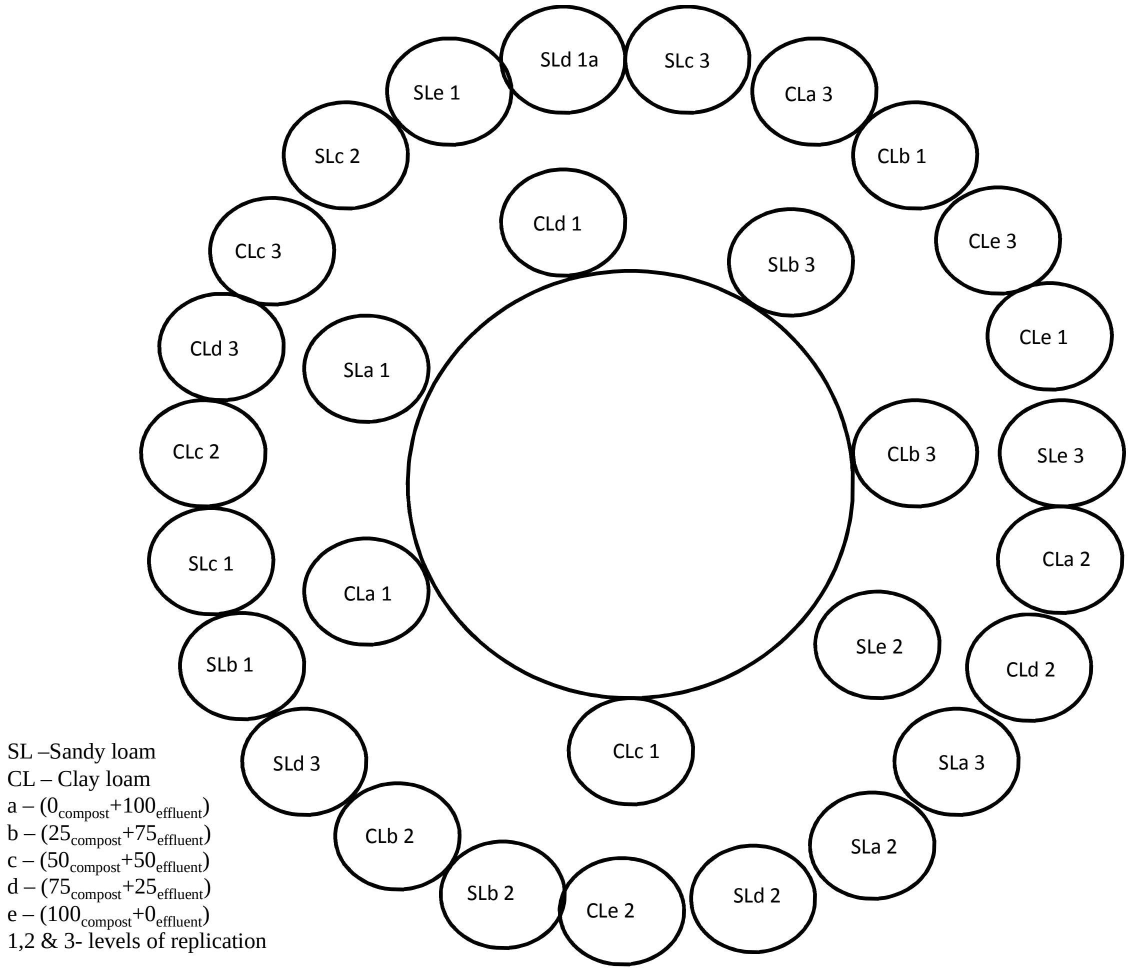


Figure C-1 Layout of the lysimeter experiment in Silsoe, Bedfordshire

Figure C-2 Lysimeter experiment in pictures

C.1.2 Experimental activities

Table C.1-1 Dates of experimental establishment and monitoring

Date	Activity
16/03/2011	Started packing soil into 200-L containers
17/03/2011	Lysimeter preparations
28 – 31 March 2011	Digging and installing the lysimeters in the ground
01-Apr-11	Planting Ryegrass
05-Apr-11	Germination has started in most of the lysimeters.
20-May-11	Installed leachate containers.
24-May-11	First effluent irrigation done
01-Jun-11	Leachate collection no 1
15th June 2011	Leachate collection no 2
21st June 2011	1st ryegrass cut
22nd June 2011	Soil sampling
05/07/2011	Leachate collection no. 3
25th July 2011	Leachate collection no. 4
09/08/2011	Leachate collection no. 5
30/08/2011	Leachate collection no. 6
02/09/2011	2nd ryegrass cut
28/09/2011	Leachate collection no. 7
10/11/2011	Leachate collection no. 8
21/12/2011	Leachate collection no. 9
20/01/2012	Leachate collection no. 10
08/03/2012	Leachate collection no. 11
29/03/2012	3rd ryegrass cut
12/04/2012	Leachate collection no. 12
10/05/2012	Leachate collection no. 13
19/06/2012	Leachate collection no. 14
26/06/2012	Leachate collection no. 15
30/07/2012	Leachate collection no. 16
30-Jul-12	4th ryegrass

Table C.1-1 presences a list of major activities carried out during the lysimeter experiment. The major activities included sewage effluent collection and irrigation and cutting of ryegrass.

C.2 Statistics

Table C.2-1 Analysis of variance for Ryegrass DM for the four cuts made for the lysimeter experiment.

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	2337275	4	584319	4.4	**0.01**
Soil type	18901335	1	18901335	141.1	**0.00**
Compost-effluent blends x Soil type	2154370	4	538592	4.0	**0.02**
Error	2277268	17	133957		
Cut	163569385	3	54523128	283.3	**0.00**
Cut x Soil type	8393096	12	699425	3.6	**0.00**
Cut x Compost-effluent blends	104712470	3	34904157	181.3	**0.00**
CUT*Compost-effluent blends*Soil type	4424425	12	368702	1.9	0.05
Error	9816348	51	192477		

Table C.2-2 Analysis of variance for total ryegrass DM yield for the lysimeter experiment

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	9349099	4	2337275	4	***0.01***
Soil type	75605341	1	75605341	141	***0.00***
Compost-effluent blends x Soil type	8617479	4	2154370	4	***0.02***
Error	9109073	17	535828		

Table C.2-3 Analysis of variance for Total NUE for all the ryegrass cuts made in the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost/effluent combination (%)	5617	4	1404	58	*0.00*
Soil type	1772	1	1772	73	*0.00*
Compost/effluent combination (%)*Soil type	1203	4	301	12	*0.00*
Error	413	17	24		

Table C.2-4 Analysis of variance for NUE for the individual ryegrass cuts made in the lysimeter experiment.

Source of variation	SS	D.o.F	MS	F	p
Compost/effluent combination (%)	1404	4	351	58	**0.00**
Soil type	443	1	443	73	**0.00**
Compost/effluent combination (%)*Soil type	301	4	75	12	**0.00**
Error	103	17	6		
CUT	8707	3	2902	248	**0.00**
CUT*Compost/effluent combination (%)	342	12	29	2	**0.01**
CUT*Soil type	5651	3	1884	161	**0.00**
CUT*Compost/effluent combination (%)*Soil type	444	12	37	3	**0.00**
Error	597	51	12		

Table C.2-5 Analysis of variance for total nitrogen uptake for all the cuts made during the lysimeter experiment.

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	279	4	70	0.36	0.84
Soil type	19127	1	19127	98.07	**0.00**
Compost-effluent blends*Soil type	1524	4	381	1.95	0.14
Error	3901	20	195		
CUT	158618	3	52873	170.39	**0.00**
CUT*Compost-effluent blends	10374	12	864	2.79	**0.00**
CUT*Soil type	15359	3	5120	16.50	**0.00**
CUT*Compost-effluent blends*Soil type	5788	12	482	1.55	0.13
Error	18618	60	310		

Table C.2-6 Analysis of variance for nitrogen uptake during the lysimeter experiment

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	1683	4	421	0.578	0.682
Soil type	67983	1	67983	93.427	**0.000**
Compost-effluent blends*Soil type	5442	4	1360	1.870	0.157
Error	13826	19	728		

Table C.2-7 Analysis of variance for phosphorous plant uptake in ryegrass cuts made during the lysimeter experiment.

Source	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	3	4	0.79	0.29	0.884
Soil type	142	1	142.21	51.36	***0.000***
Compost-effluent blends*Soil type	11	4	2.69	0.97	0.448
Error	50	18	2.77		
CUT	501	3	166.87	45.79	***0.000***
CUT*Compost-effluent blends	26	12	2.13	0.58	0.845
CUT*Soil type	257	3	85.52	23.47	***0.000***
CUT*Compost-effluent blends*Soil type	22	12	1.81	0.50	0.908
Error	197	54	3.64		

Table C.2-8 Analysis of variance for total nitrogen in plant material matter during the lysimeter experiment

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	0.65	4	0.16	1.73	0.19
Soil type	4.93	1	4.93	52.50	***0.00***
Compost-effluent blends*Soil type	0.23	4	0.06	0.62	0.65
Error	1.69	18	0.09		
CUT	22.18	3	7.39	112.20	***0.00***
CUT*Compost-effluent blends	0.78	12	0.07	0.99	0.47
CUT*Soil type	3.94	3	1.31	19.92	***0.00***
CUT*Compost-effluent blends*Soil type	0.54	12	0.05	0.68	0.76
Error	3.56	54	0.07		

Table C.2-9 Analysis of variance for soil pH for soils samples after the first cut for 0 to 10 cm and 10 to 50 cm

Source	SS	D.O.F	MS	F	p
Compost-effluent blends	0.1	4	0.04	4	***0.02***
Soil type	18.1	1	18.12	1868	***0.00***
Compost-effluent blends*Soil type	0.1	4	0.04	4	***0.02***
Error	0.2	17	0.01		
DEPTH	0.0	1	0.01	1	0.27
DEPTH*Compost-effluent blends	0.1	4	0.02	4	***0.02***
DEPTH*Soil type	0.0	1	0.02	4	0.07
DEPTH*Compost-effluent blends*Soil type	0.1	4	0.02	4	***0.02***
Error	0.1	17	0.01		

Table C.2-10 Analysis of variance for soil pH for soils samples after the last cut for 0 to 10 cm and 10 to 50 cm

Source	SS	D.o.F	MS	F	p
Compost-effluent blends	0.2	4	0.1	1.5	0.25
Soil type	9.6	1	9.6	239.9	***0.00***
Compost-effluent blends*Soil type	0.3	4	0.1	1.6	0.22
Error	0.7	17	0.0		
DEPTH	0.1	1	0.1	3.6	0.07
DEPTH*Compost-effluent blends	0.2	4	0.0	2.2	0.12
DEPTH*Soil type	0.1	1	0.1	5.2	***0.04***
DEPTH*Compost-effluent blends*Soil type	0.2	4	0.1	3.3	***0.03***
Error	0.3	17	0.0		

Table C.2-11 Analysis of variance for soil pH for soils samples after the first and last cut for 0 to 10 cm

Source	SS	D.o.F	MS	F	p
Compost-effluent blends	0.04	4	0.01	0.4	0.82
Soil type	11.89	1	11.89	466.2	***0.00***
Compost-effluent blends*Soil type	0.12	4	0.03	1.1	0.37
Error	0.43	17	0.03		
TIME	0.53	1	0.53	25.4	***0.00***
TIME*Compost-effluent blends	0.04	4	0.01	0.5	0.74
TIME*Soil type	0.44	1	0.44	20.7	***0.00***
TIME*Compost-effluent blends*Soil type	0.01	4	0.00	0.1	0.99
Error	0.36	17	0.02		

Table C.2-12 Analysis of variance for soil pH for soils samples after the first and last cut for 10 to 50 cm

Source	SS	D.o.F	MS	F	p
Compost-effluent blends	0.53	4	0.13	6	***0.00***
Soil type	15.26	1	15.26	729	***0.00***
Compost-effluent blends*Soil type	0.56	4	0.14	7	***0.00***
Error	0.36	17	0.02		
TIME	1.16	1	1.16	171	***0.00***
TIME*Compost-effluent blends	0.02	4	0.01	1	0.55
TIME*Soil type	0.25	1	0.25	36	***0.00***
TIME*Compost-effluent blends*Soil type	0.05	4	0.01	2	0.17
Error	0.12	17	0.01		

Table C.2-13 Analysis of variance for extractable P for soils samples after the first and last cut for 0 to 10 cm

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	19.37	4	4.84	1.562	**0.23**
Soil type	42.70	1	42.70	13.772	***0.00***
Compost-effluent blends*Soil type	42.86	4	10.71	3.456	***0.03***
Error	58.91	19	3.10		
Time	150.58	1	150.58	37.412	***0.00***
Time x compost-effluent blends	11.41	4	2.85	0.709	0.60
Time x soil type	5.60	1	5.60	1.390	0.25
Time x Compost-effluent blends x Soil type	0.49	4	0.12	0.030	1.00
Error	76.47	19	4.02		

Table C.2-14 Analysis of variance for extractable P for soils samples after the first and last cut for 10 to 50 cm

Source of variation	**SS**	**D.o.F**	**MS**	**F**	**p**
Compost-effluent blends	27.3	4	6.8	2.32	0.09
Soil type	49.1	1	49.1	16.71	***0.00***
Compost-effluent blends*Soil type	34.4	4	8.6	2.92	***0.05***
Error	55.9	19	2.9		
Time	0.7	1	0.7	0.31	0.58
Time x compost-effluent blends	6.6	4	1.6	0.70	0.60
Time x soil type	1.4	1	1.4	0.58	0.46
Time x compost-effluent blends x Soil type	2.3	4	0.6	0.24	0.91
Error	44.9	19	2.4		

Table C.2-15 Analysis of variance for extractable P for soils samples after the last cut for 0 to 10 cm and 10 to 50 cm.

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	9	4	2	0.6	0.68
Soil type	54	1	54	13.7	***0.00***
Compost-effluent blends*Soil type	13	4	3	0.9	0.51
Error	75	19	4		
Depth	503	1	503	85.7	***0.00***
Depth x compost-effluent blends	8	4	2	0.3	0.85
Depth x soil type	2	1	2	0.4	0.54
Depth x Compost-effluent blends x Soil type	17	4	4	0.7	0.58
Error	111	19	6		

Table C.2-16 Analysis of variance for extractable P for soils samples after the first cut for 0 to 10 cm and 10 to 50 cm.

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	5	4	1.3	2	0.20
Soil type	38	1	38.1	51	***0.00***
Compost-effluent blends*Soil type	26	4	6.5	9	***0.00***
Error	14	19	0.8		
Depth	121	1	121.3	66	***0.00***
Depth x compost-effluent blends	42	4	10.6	6	***0.00***
Depth x soil type	4	1	4.0	2	0.16
Depth x Compost-effluent blends x Soil type	23	4	5.8	3	***0.04***
Error	35	19	1.9		

Table C.2-17 Analysis of variance for cumulative nitrate leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	3535	4	884	5	***0.01***
Soil type	10114	1	10114	53	***0.00***
Compost-effluent blends*Soil type	3389	4	847	4	***0.01***
Error	3619	19	190		
TIME	824	13	63	15	***0.00***
TIME*Compost-effluent blends	360	52	7	2	***0.01***
TIME*Soil type	590	13	45	11	***0.00***
TIME*Compost-effluent blends*Soil type	324	52	6	1	***0.03***
Error	1043	247	4		

Table C.2-18 Analysis of variance for cumulative total dissolved nitrogen leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	5958936	4	1489734	5	***0.01***
Soil type	22684654	1	22684654	72	***0.00***
Comp-effluent blends*Soil type	6356603	4	1589151	5	***0.01***
Error	5976691	19	314563		
TIME	5042956	8	630370	51	***0.00***
TIME*Compost-effluent blends	1401160	32	43786	4	***0.00***
TIME*Soil type	3494762	8	436845	35	***0.00***
TIME*Compost-effluent blends*Soil type	1473134	32	46035	4	***0.00***
Error	1872266	152	12318		

Table C.2-19 Analysis of variance for cumulative phosphorous leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	0.18	4	0.04	4.34	***0.01***
Soil type	0.04	1	0.04	3.66	0.07
Compost-effluent blends*Soil type	0.06	4	0.01	1.43	0.26
Error	0.19	18	0.01		
TIME	0.05	12	0.00	40.21	***0.00***
TIME*Compost-effluent blends	0.01	48	0.00	2.94	***0.00***
TIME*Soil type	0.01	12	0.00	4.57	***0.00***
TIME*Compost-effluent blends*Soil type	0.01	48	0.00	1.64	***0.01***
Error	0.02	216	0.00		

Table C.2-20 Analysis of variance for nitrate concentration leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	4090	4	1023	13	***0.00***
Soil type	8414	1	8414	111	***0.00***
Compost-effluent blends*Soil type	4026	4	1007	13	***0.00***
Error	1141	15	76		
TIME	8580	15	572	21	***0.00***
TIME*Compost-effluent blends	9380	60	156	6	***0.00***
TIME*Soil type	9371	15	625	23	***0.00***
TIME*Compost-effluent blends*Soil type	8803	60	147	5	***0.00***
Error	6192	225	28		

Table C.2-21 Analysis of variance for phosphorous concentration leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	0.31	4	0.08	3.08	***0.05***
Soil type	0.09	1	0.09	3.71	0.07
Compost-effluent blends*Soil type	0.06	4	0.01	0.57	0.69
Error	0.40	16	0.03		
TIME	2.41	13	0.19	9.07	***0.00***
TIME*Compost-effluent blends	1.69	52	0.03	1.59	***0.01***
TIME*Soil type	1.26	13	0.10	4.74	***0.00***
TIME*Compost-effluent blends*Soil type	1.44	52	0.03	1.35	0.07
Error	4.25	208	0.02		

Table C.2-22 Analysis of variance for ammonium concentration leaching for the lysimeter experiment

Source of variation	SS	D.o.F	MS	F	p
Compost-effluent blends	0.05	4	0.01	0.48	0.75
Soil type	0.01	1	0.01	0.33	0.58
Compost-effluent blends*Soil type	0.12	4	0.03	1.12	0.40
Error	0.27	10	0.03		
TIME	0.96	15	0.06	4.34	***0.00***
TIME*Compost-effluent blends	1.59	60	0.03	1.80	***0.00***
TIME*Soil type	0.44	15	0.03	1.99	***0.02***
TIME*Compost-effluent blends*Soil type	1.16	60	0.02	1.32	0.09
Error	2.20	150	0.01		

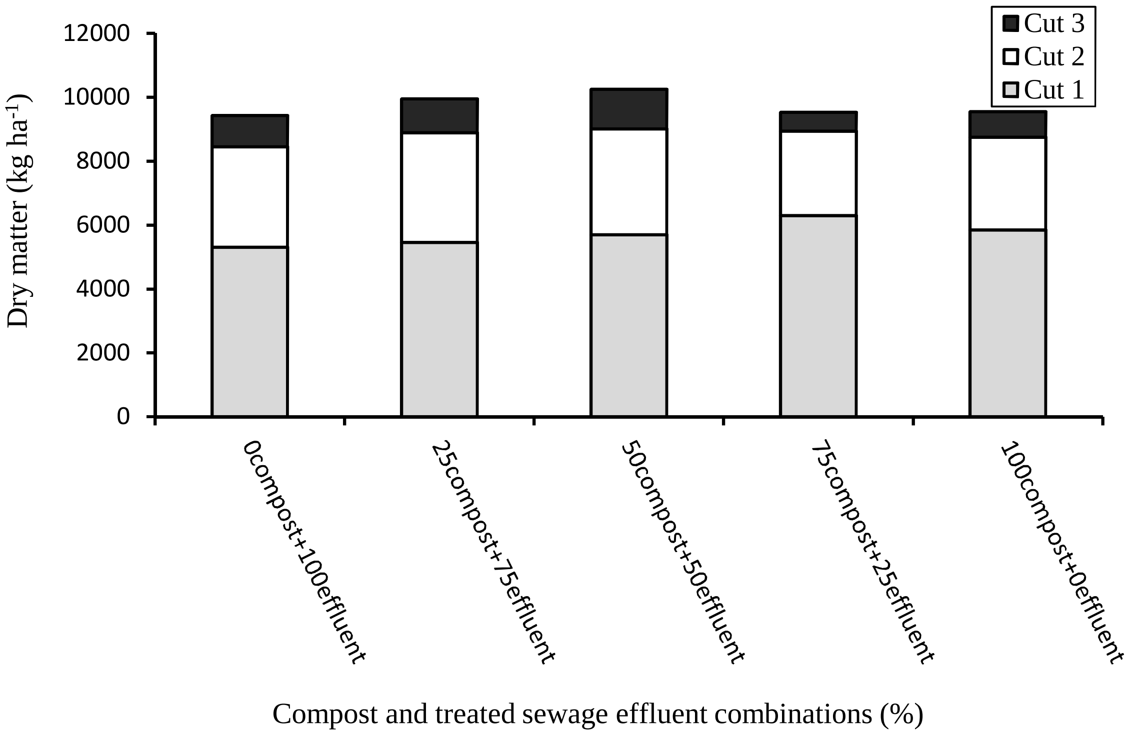


Figure C-3 Ryegrass dry matter harvested per cut in sandy loam in the lysimeter experiment.

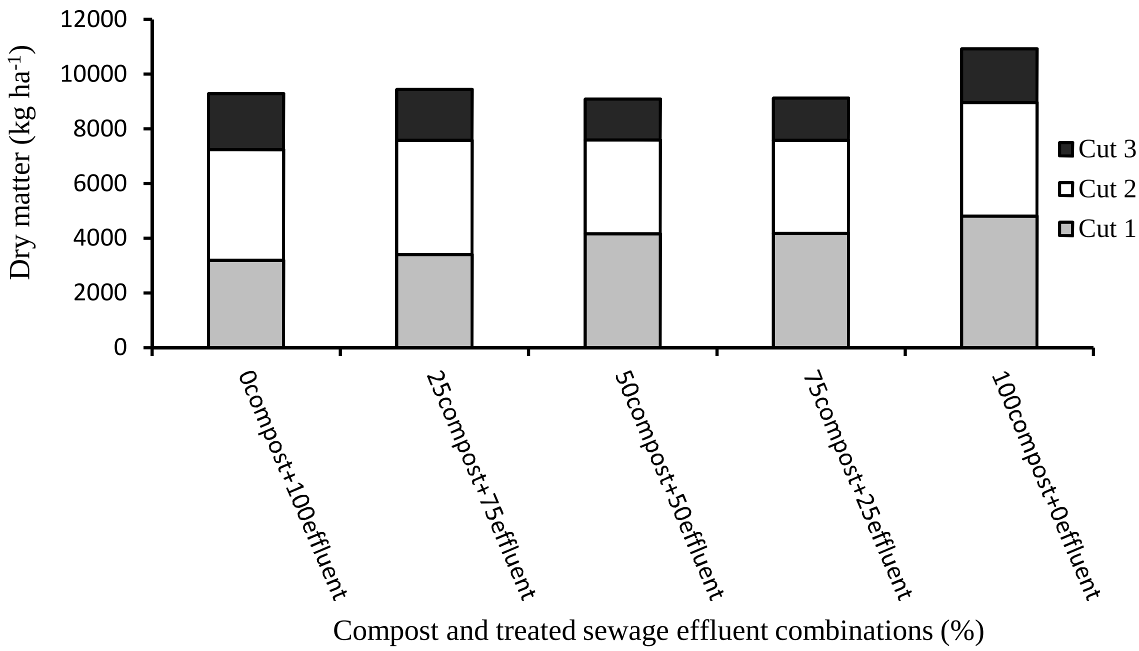
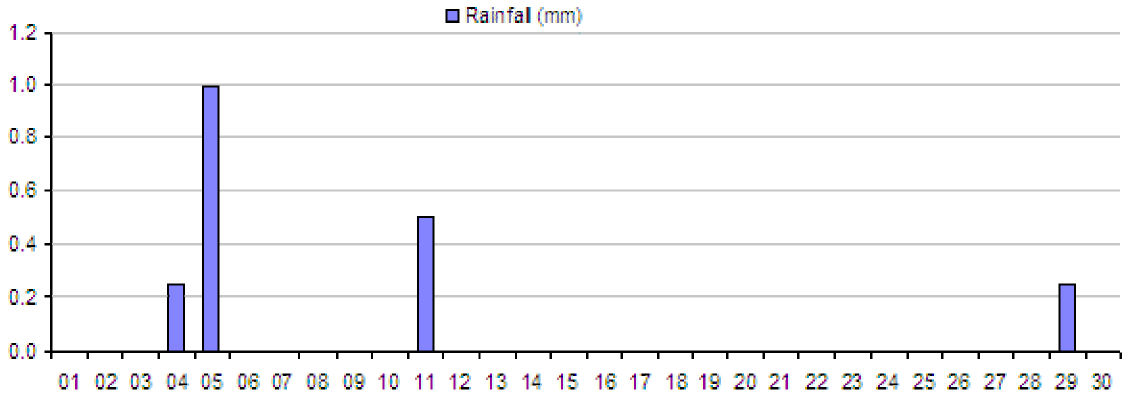


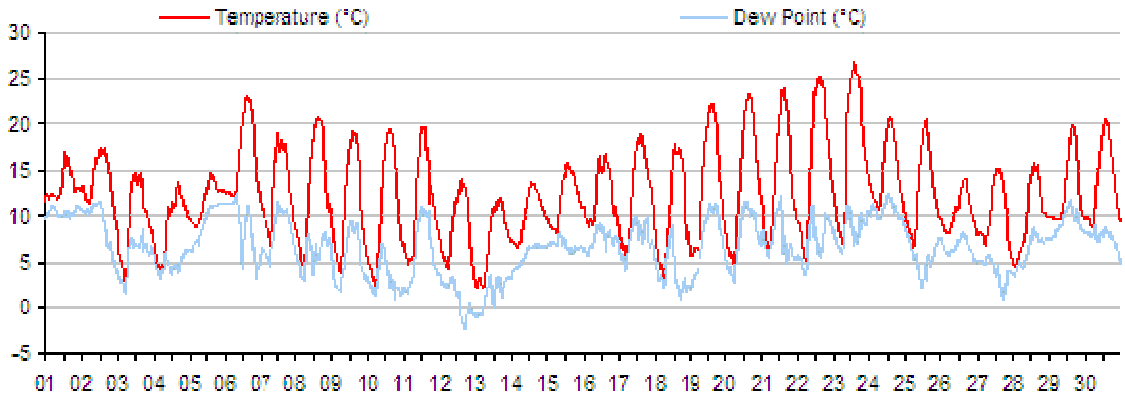
Figure C-4 Ryegrass dry matter harvested per cut in clay loam in the lysimeter experiment.

C.3 Climatic data

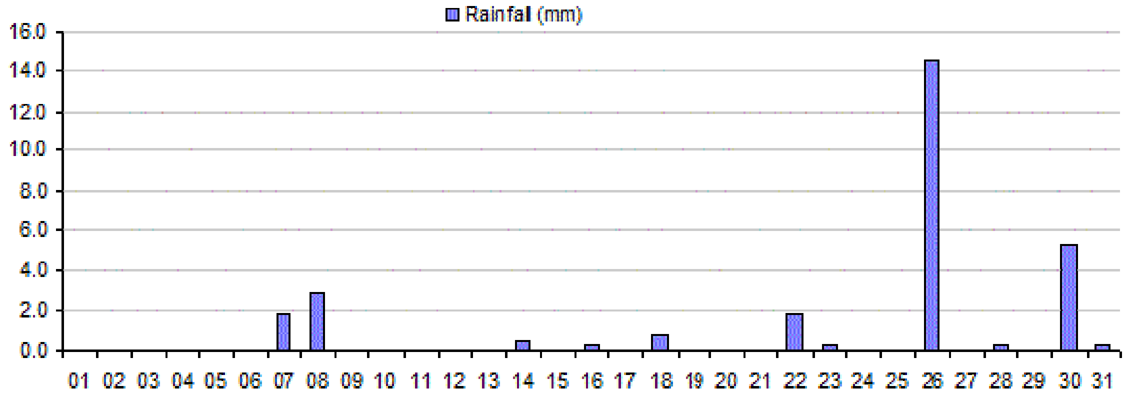
Selected climatic data (**Source: Clifton weather, Bedfordshire, UK**)

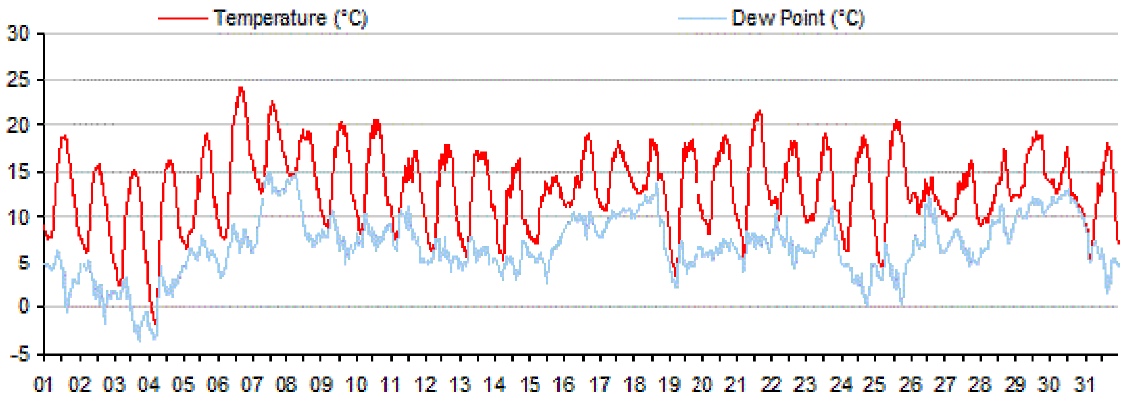
April 2011



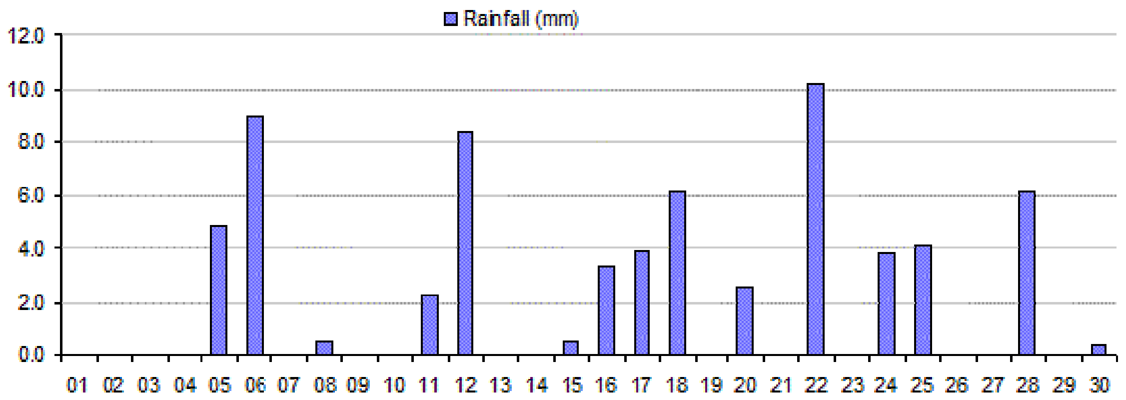


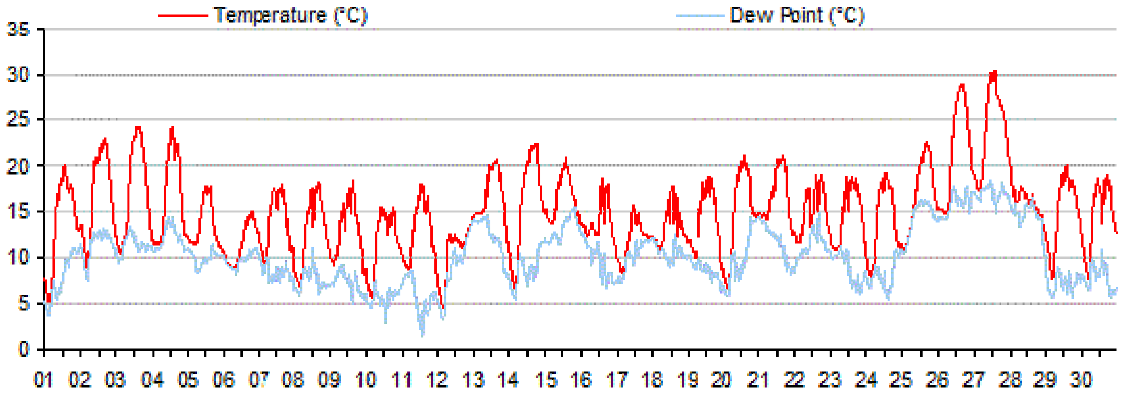
May 2011



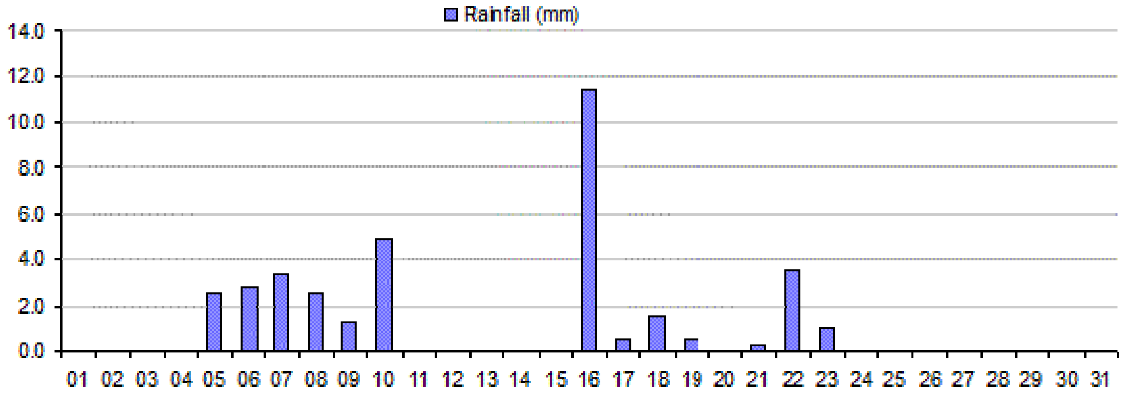

Temperature (°C)
Dew Point (°C)
30
25
20
15
10
5
0
-5
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

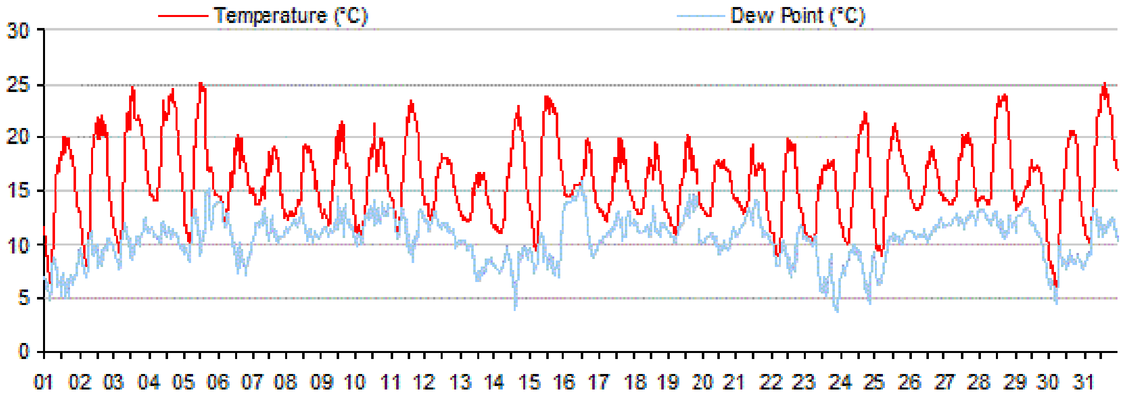
June 2011


Rainfall (mm)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

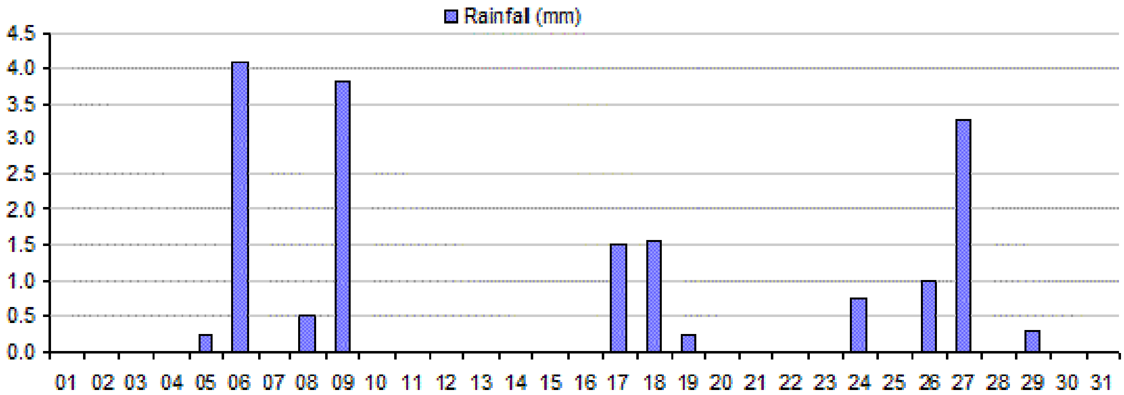

Temperature (°C)
Dew Point (°C)
35
30
25
20
15
10
5
0
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

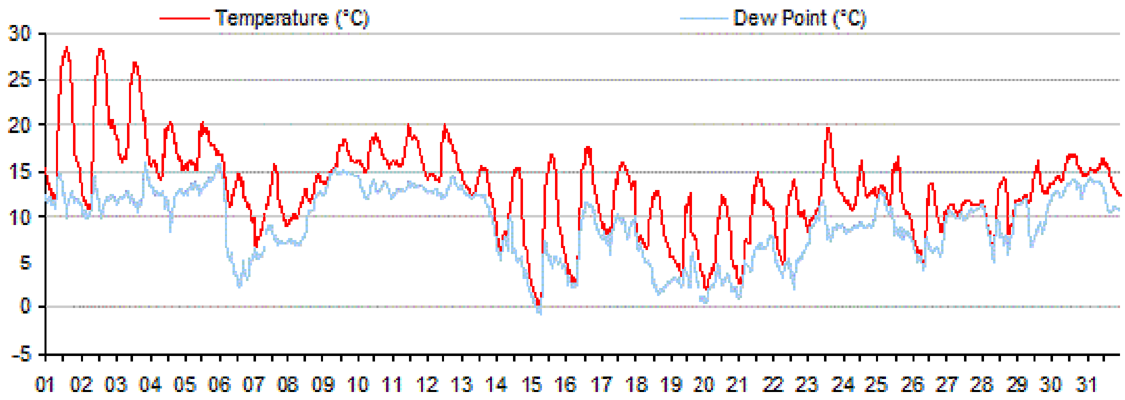
August 2011



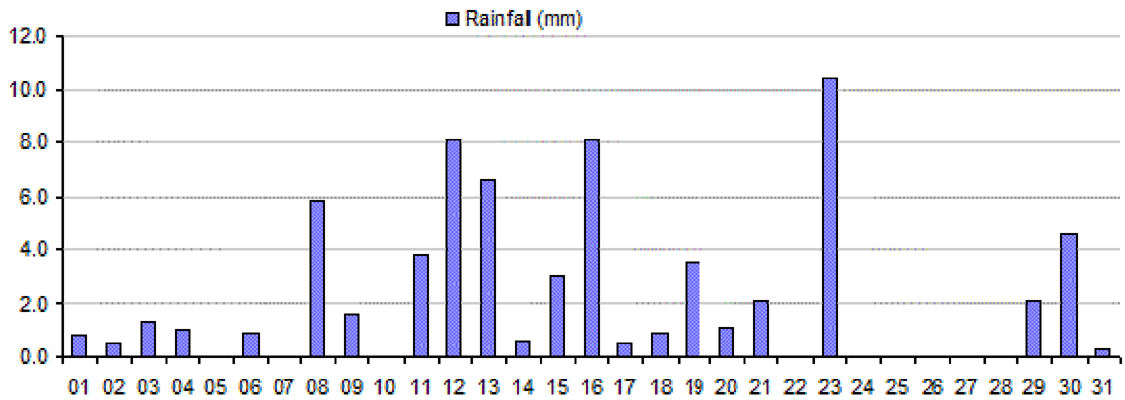


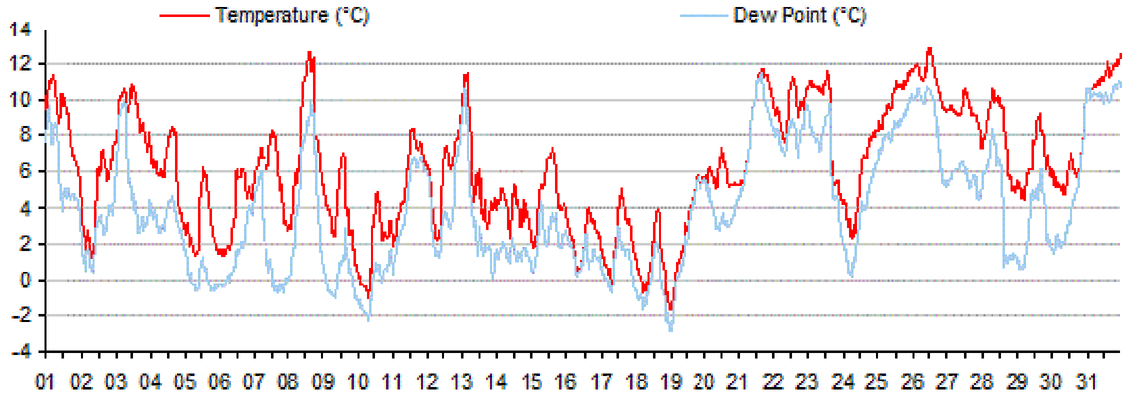
October 2011



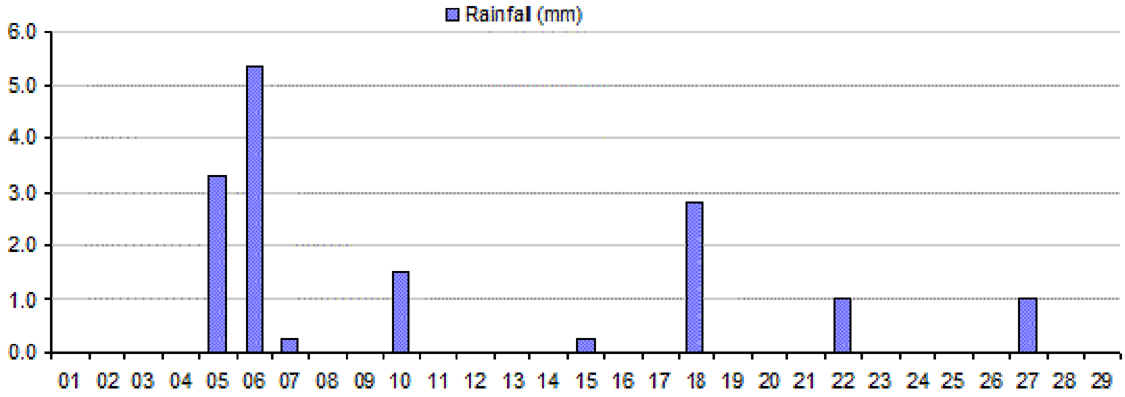


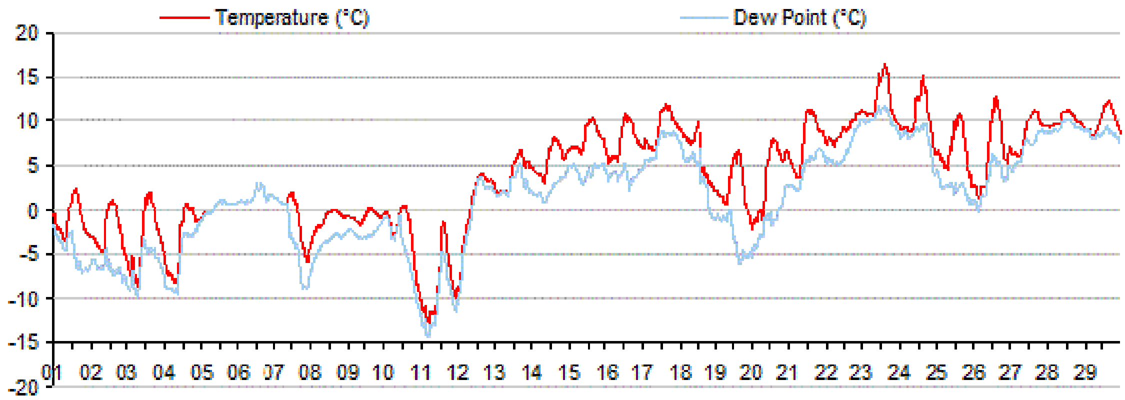
December 2011



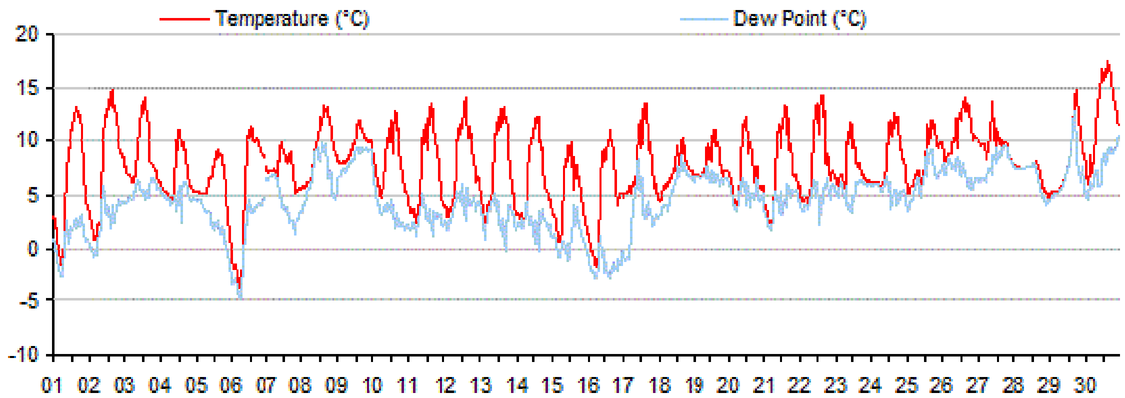


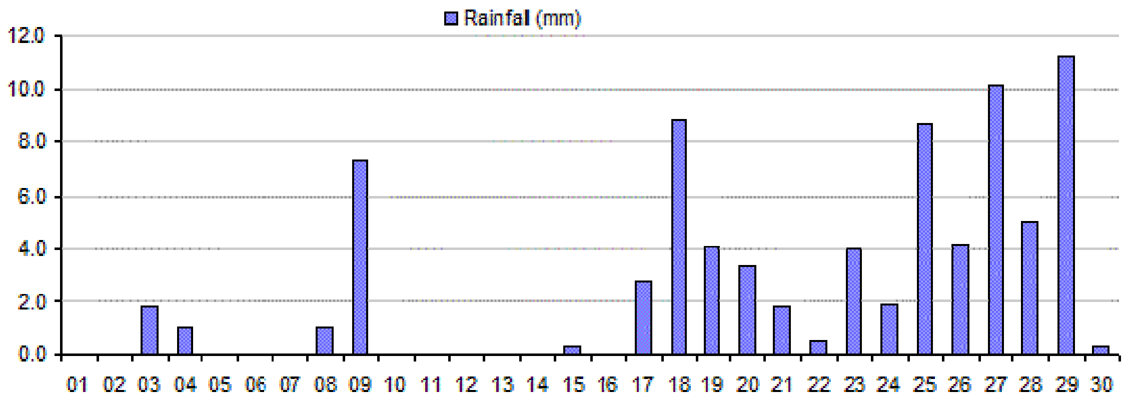
February 2012



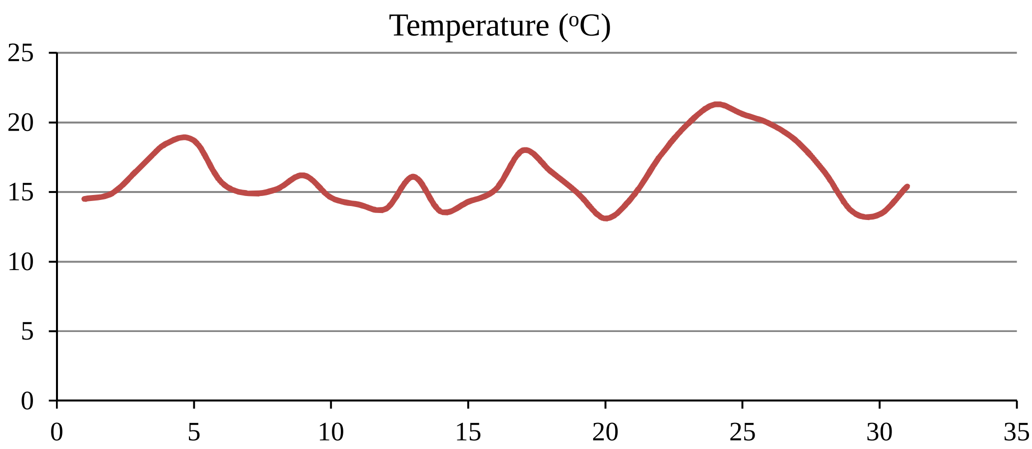


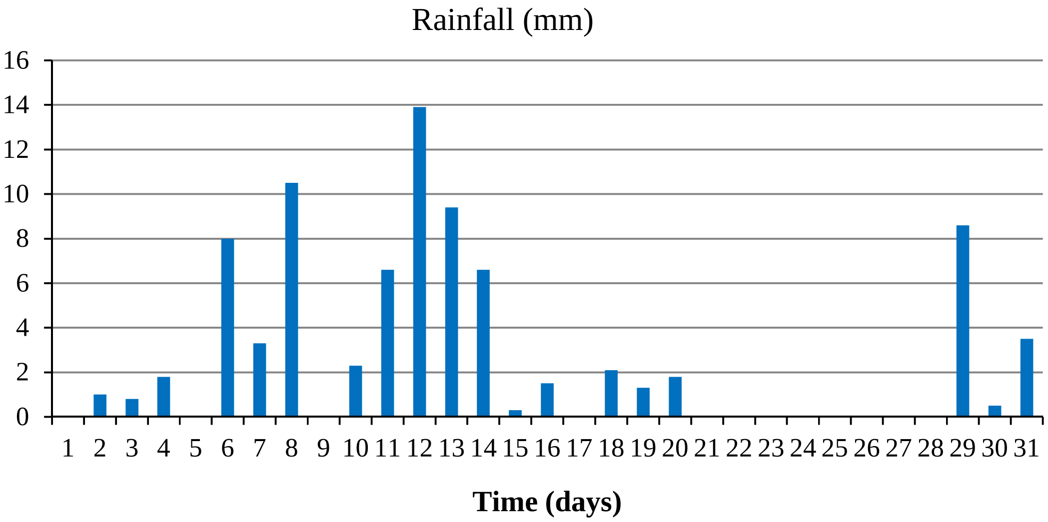
April 2012




Rainfal (mm)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

July 2012


Temperature (°C)
25
20
15
10
5
0
0 5 10 15 20 25 30 35


Rainfall (mm)
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Time (days)

C.4 Leachate volume

Table C.4-1 Mean leachate volume (mm) collected in clay loam soil in the lysimeter experiment

	Leachate volume (mm) – Clay loam				
Date	**$0_{compost}+100_{effluent}$**	**$25_{compost}+75_{effluent}$**	**$50_{compost}+50_{effluent}$**	**$75_{compost}+25_{effluent}$**	**$100_{compost}+0_{effluent}$**
01/06/2011	7.2	2.8	9.5	9.2	3.5
15/06/2011	6.6	2.5	3.1	6.6	2.8
05/07/2011	2.6	1.1	3.9	3.0	0.2
25/07/2011	16.3	10.5	15.8	19.6	10.7
09/08/2011	13.2	10.7	16.0	18.7	12.6
30/08/2011	8.4	2.9	8.7	8.1	3.0
28/09/2011	8.0	3.8	10.5	7.9	4.1
11/11/2011	1.6	4.7	6.2	3.4	6.0
21/12/2011	9.9	9.3	15.0	12.5	7.2
20/01/2012	9.3	6.7	12.9	11.6	5.6
09/03/2012	9.5	3.1	10.4	8.8	3.4
12/04/2012	4.6	2.5	4.1	1.5	0.7
10/05/2012	14.2	9.5	15.5	11.6	8.4
19/06/2012	3.2	1.7	3.7	3.8	3.1
26/06/2012	4.7	2.6	6.9	1.5	1.4
30/07/2012	13.0	7.7	3.9	2.5	1.7
Total	**132.4**	**81.8**	**146.0**	**130.3**	**74.3**

Table C.4-2 Mean leachate volume (mm) collected in sandy loam soil in the lysimeter experiment

	Leachate volume (mm) – Sandy loam				
Date	**$0_{compost}$+$100_{effluent}$**	**$25_{compost}$+$75_{effluent}$**	**$50_{compost}$+$50_{effluent}$**	**$75_{compost}$+$25_{effluent}$**	**$100_{compost}$+$0_{effluent}$**
01/06/2011	2.2	0.2	1.2	1.0	2.1
15/06/2011	0.0	0.0	0.0	0.0	0.0
05/07/2011	0.1	0.0	0.0	0.0	0.0
25/07/2011	3.9	2.4	8.1	6.2	4.2
09/08/2011	0.4	0.8	1.6	2.6	5.0
30/08/2011	8.7	0.0	9.5	0.5	1.9
28/09/2011	11.0	0.4	3.5	0.5	2.5
11/11/2011	0.3	0.0	0.6	1.1	0.1
21/12/2011	2.0	0.8	2.6	2.4	2.1
20/01/2012	4.0	9.7	7.4	14.1	14.2
09/03/2012	12.6	8.9	19.1	18.2	17.7
12/04/2012	4.9	6.2	7.9	5.9	6.4
10/05/2012	13.6	13.8	17.2	10.9	14.2
19/06/2012	10.6	6.5	7.3	11.9	11.8
26/06/2012	3.5	4.6	3.5	7.0	4.5
30/07/2012	13.4	19.1	6.0	11.8	9.4
Total	**91.1**	**73.5**	**95.5**	**94.1**	**96.2**

C.5 Ryegrass dry matter

Table C.5-1 Ryegrass DM yield (kg ha^{-1}) from the lysimeter experiment

	$(0_{compost}+100_{effluent})$	$(25_{compost}+75_{effluent})$	$(50_{compost}+50_{effluent})$	$(75_{compost}+25_{effluent})$	$(100_{compost}+0_{effluent})$
Clay loam					
Cut 1	3195	3400	4161	4179	4808
Cut 2	4050	4175	3434	3401	4155
Cut 3	2044	1864	1491	1551	1961
Cut 4	5513	6148	4706	5701	4975
Sandy loam					
Cut 1	5306	5462	5702	6302	5847
Cut 2	3141	3429	3310	2637	2900
Cut 3	987	1061	1241	593	805
Cut 4	2238	2210	2516	1659	1534

Appendix D Conferences

Oral presentations

Chipula, G., Sakrabani, R., Hess, T., (2011). *"The interaction between STSE and compost on nitrogen dynamics in soil"*. Young Scientist's conference, University of York, 23 - 24th March 2011.

Chipula, G., Sakrabani, R., Hess, T., (2012). "*Nutrient integration and its impact on soil properties and nutrient uptake."* Environment DTC Research Student Conference. 23rd November, 2011, Cranfield, UK.

Poster presentations

Chipula, G., Sakrabani, R., Hess, T., (2011). *"The influence of the interaction of treated sewage effluent and compost on the dynamics of nitrogen in arable soil."* 6th International Symposium of Interactions of Soil Minerals with Organic Components and Microorganisms Commission. 26 June – 1st July, 2011, Montpellier, France

Chipula, G., Sakrabani, R., Hess, T., (2012). "*Nitrogen kinetics and mineralisation in a compost-sewage effluent amended soil."* 4th International Congress of EUROSOIL, 2nd – 7th July, 2012.